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Excitatory amino acid neurotoxicity and modulation of glutamate receptor expression in organotypic brain slice cultures

Review Article

J. Zimmer[1,2], **B. W. Kristensen**[1,2], **B. Jakobsen**[2], and **J. Noraberg**[1,2]

[1]NeuroScreen, Ltd. and
[2]Department of Anatomy & Neurobiology, Southern Denmark-Odense University, Odense, Denmark

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Summary. Using organotypic slice cultures of hippocampus and cortex-striatum from newborn to 7 day old rats, we are currently studying the excitotoxic effects of kainic acid (KA), AMPA and NMDA and the neuroprotective effects of glutamate receptor blockers, like NBQX. For detection and quantitation of the induced neurodegeneration, we have developed standardized protocols, including – a) densitometric measurements of the cellular uptake of propidium iodide (PI), – b) histological staining by Flouro-Jade, – c) lactate dehydrogenase (LDH) release to the culture medium, – d) immunostaining for microtubulin-associated protein 2, and – e) general and specific neuronal and glial cell stains. The results show good correlation between the different markers, and are in accordance with results obtained *in vivo*. Examples presented in this review will focus on the use of PI uptake to monitor the excitotoxic effects of – a) KA and AMPA (and NMDA) in hippocampal slice cultures, and – b) KA and AMPA in corticostriatal slice cocultures, with demonstration of differentiated neuroprotective effects of NBQX in relation to cortex and striatum and KA and AMPA. A second set of studies include modulation of hippocampal KA-induced excitotoxicity and KA-glutamate receptor subunit mRNA expression after long-term exposure to low, non-toxic doses of KA and NBQX.

We conclude that organotypic brain slice cultures, combined with standardized procedures for quantitation of cell damage and receptor subunit changes is of great potential use for studies of excitotoxic, glutamate receptor-induced neuronal cell death, receptor modulation and related neuroprotection.

Keywords: Amino acids – Brain slice cultures – Hippocampus – Striatum – Cerebral cortex – Kainic acid – AMPA – NBQX – Propidium iodide

Introduction

Slices of developing, postnatal brain tissue can be grown in tissue culture for weeks, or sometimes even months (Gähwiler, 1984, 1988; Stoppini et al., 1991; Bergold and Casaccia Bonnefil, 1997; Gähwiler et al., 1997; Thibaud et al., 1997; Jahnsen et al., 1999) (Fig. 1). With preservation of the basic cellular and connective organization of the donor brain regions the slice cultures provide an easily accessible experimental model for studies of toxic, degenerative and plastic regenerative or developmental changes in the brain. The easy access to the tissue and a better control of experimental conditions, combined with preservation of the basic cellular composition and synaptic circuitry, do in many experimental situations make organotypic brain slice cultures preferable to both live animals and dissociated neuronal cultures.

Hippocampal slice cultures in particular, are well characterised with regard to their neuronal and connective organisation (Fig. 2) and electrophysiological properties (Gähwiler, 1984, 1988; Zimmer and Gähwiler, 1984, 1987; Frotscher et al., 1990; Finsen et al., 1992; Torp et al., 1992; Schousboe et al., 1993; Tønder et al., 1993; Gähwiler et al., 1997), but also slice cultures of the dopaminergic nigrostriatal system (Østergaard et al., 1990, 1991) and cortex-striatum slice cultures are well studied (Østergaard, 1993; Østergaard et al., 1995; Plenz and Aertsen, 1996ab; Plenz and Kitai, 1996, 1998) for characterization of their basic cellular, connective and functional organization, or for experimental manipulation, including application of neurotrophic factors (Østergaard et al., 1996; Sautter et al., 1998; Meyer et al., 1999; Dahl-Jørgensen et al., 1999) or potentially toxic compounds (Whetsell and Schwarcz, 1983; Kristensen et al., 1999).

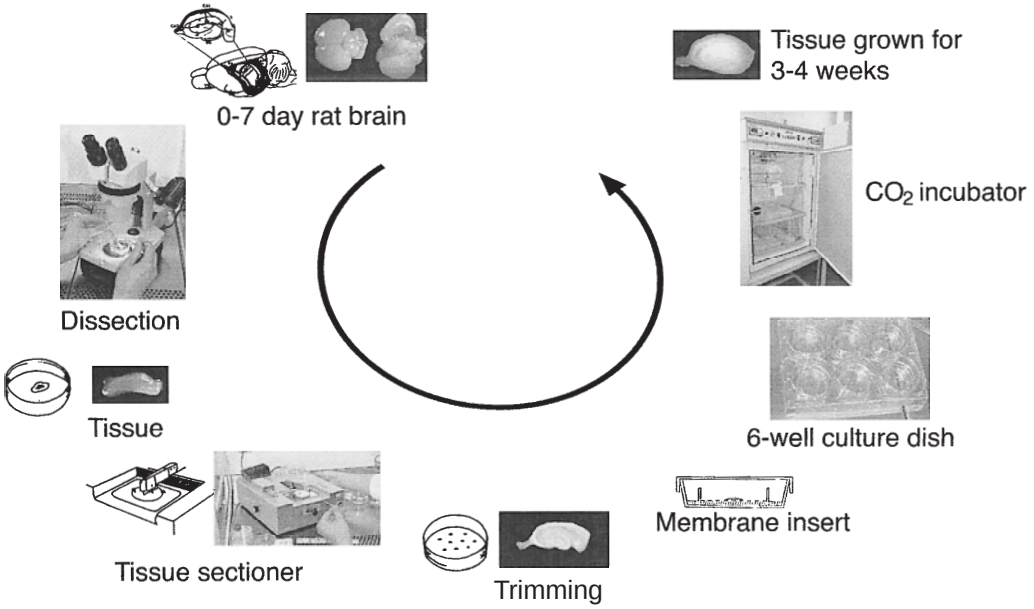

Fig. 1. Preparation of hippocampal brain slice cultures according to the insert or interface membrane method. For proper description of dissection and explantation of tissue, composition of media etc., see original publications, like Noraberg et al. (1999)

Use of hippocampal slice cultures as models for neurodegeneration

Organotypic slice cultures of hippocampal tissue have in the past few years been used to study neuronal death induced by a variety of conditions or compounds, like hypoxia (Pringle et al., 1997a), hypoglycemia (Tasker et al., 1992), a combination of the two to mimic cerebral ischemia (Hsu et al., 1994; Vornov et al., 1994; Newell et al., 1995; Pringle et al., 1997b), nitric oxide (Bahr, 1995), oxidative stress (Wilde et al., 1997; Vornov et al., 1998), excitotoxins (Rimvall et al., 1987; Vornov et al., 1991; Sakaguchi et al., 1997), colchicine (Newell et al., 1993), trimethyltin (Noraberg et al., 1998), β-amyloid peptide (Bruce et al., 1996) and organic solvents, like ketones and ethanol (Noraberg et al., 1997; Collins et al., 1998; Noraberg and Zimmer, 1998; Thomas et al., 1998). The same or corresponding studies have tested the possibilities for application and effects of neuroprotective agents, like free radical scavengers (Bruce et al., 1996), neurotrophic factors (Nakagami et al., 1997; Pringle et al., 1996) and glutamate receptor blockers (Tasker et al., 1992; Pozzo Miller et al., 1994; Vornov et al., 1994; Gähwiler et al., 1997; Pringle et al., 1997b; Sagagushi et al., 1997).

Propidium iodide and other markers for neurodegeneration

Many of the slice culture studies, inducing hippocampal neurodegeneration, have used cellular uptake of the fluorescent dye propidium iodide (PI) as a marker for dead or dying cells. PI (3,8-Diamino-5-(3-(diethylmethylamino) propyl)-6-phenyl phenanthridinium diiodide; Sigma, Cat. No. P4170) is a very stable flourescent dye, absorbing blue-green light (493 nm) with red

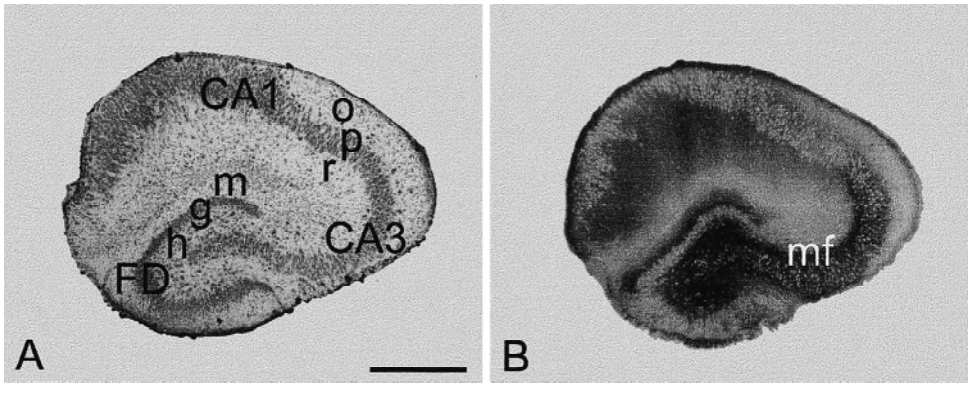

Fig. 2. Histological organization of 4 week old hippocampal slice culture, from 7 day old donor rat. **A** Toluidine blue stained cryostat section, showing the organotypic appearance of cell and neuropil layers, including fascia dentata (*FD*) with granule cell layer (*g*), molecular layer (*m*), the dentate hilus or CA4 (*h*), the hippocampal subfields CA3 and CA1 with pyramidal cell layers (*p*), str. radiatum (*r*) and str. oriens (*o*). **B** Adjacent Timm stained section with characteristic, densely stained, zinc-rich mossy fiber terminals in the dentate hilus, and extending as the mossy fiber layer (*mf*) along the pyramidal layer of CA3 to the CA3-CA1 border. Scale bar: 500 μm

fluorescence (630 nm). As a polar compound it cannot directly pass through the intact cell membrane, but it enters dead or dying cells, where it binds to DNA with an intensified, brightly red fluorescence (Macklis and Madison, 1990). PI is basically non-toxic to neurons (Hsu et al., 1994; Pozzo Miller et al., 1994; personal observations) and has been used as an indicator of neuronal membrane integrity (Vitale et al., 1993). A survey of the studies, that have used PI uptake as a marker for neuronal degeneration does, however, show considerable variability with regard to the concentration of PI, exposure time and recording of the PI uptake, just as other studies have questioned the use of PI uptake alone as a reliable marker for dying cells (Bevensee et al., 1995; Lizard et al., 1995). As part of our ongoing studies of neurodegeneration and neuroprotection in brain slice cultures, we therefore found it highly essential to establish a standardized PI protocol. Having done so (Noraberg et al., 1999), we combine the presence of a constant dose (2μM) of PI in the culture medium with digital, flourescence micrograph recordings of the fluorescent dye uptake in the cultures before ("control") and during exposure to the potential toxins, as well as after killing of all neurons ("maximal neuronal uptake"). Using the standardized protocol we obtain quantitative, absolute and relative data for the PI uptake and neuronal damage, which allow proper testing of the reproducibility of the PI uptake and comparison with other traditional and new markers for neurodegeneration.

Current use of PI and other markers in hippocampal slice cultures

Both in the methodological study (Noraberg et al., 1999) and other parallel and ongoing experimental studies (Noraberg et al., 1998; Jakobsen et al., 1999), use of the standardized PI uptake protocol on hippocampal slice cultures has resulted in very high reproducibility of data between experiments, and good correlation between the PI uptake and – 1) the distribution and density of histological staining with Fluoro-Jade (FJ), a newly developed fluorescent stain for degenerating neurons (Schmued et al., 1997) (Fig. 3), – 2) the amounts of lactate dehydrogenase (LDH) in the culture medium, due to efflux of this cytosolic enzyme from damaged or dead cells (Koh and Choi, 1987; Vassault, 1993), – 3) cell loss observed after Nissl cell staining, – 4) loss of immunohistochemical staining for microtubule-associated protein 2 (MAP2) and – 5) changes in the Timm sulphide silver staining normally visualizing Zn-containing terminals (Zimmer and Gähwiler, 1984, 1987).

In conjunction with ongoing studies of glutamate receptor ligand bindings in hippocampal slice cultures (see Fig. 4 for binding of ^{3}H-AMPA), we have also been able to use the PI regional uptakes to calculate EC_{50} values for the glutamate agonist AMPA (2-amino-3-hydroxy-5-methyl-4-isoxazole propionate) for the entire hippocampal culture (Fig. 5) and for the different hippocampal subfields.

The differential susceptibility of the individual hippocampal subfields to different glutamate receptor agonists can, in addition to the KA-induced, characteristic and *in vivo*-like degeneration in CA3 (Fig. 3), also be illustrated as quantitative differences in PI uptake. Hippocampal slice cultures exposed

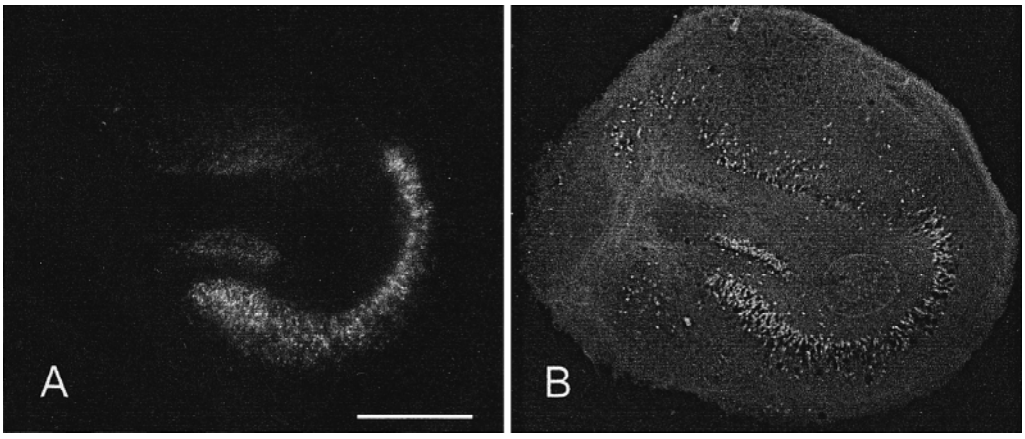

Fig. 3. Kainic acid-induced degeneration of CA3 pyramidal cells in hippocampal slice culture, demonstrated by **A** Cellular uptake of PI, recorded by flourescence microscopy of live culture after exposure to 10 μM kainic acid for 48 h followed by 48 h in normal medium; **B** Fluoro-Jade staining of cryostat section of same culture. The preferential lesioning of CA3 pyramidal cell by KA in the slice cultures mimics the corresponding lesion *in vivo* (Nadler et al., 1980). Scale bar: 0.5 mm

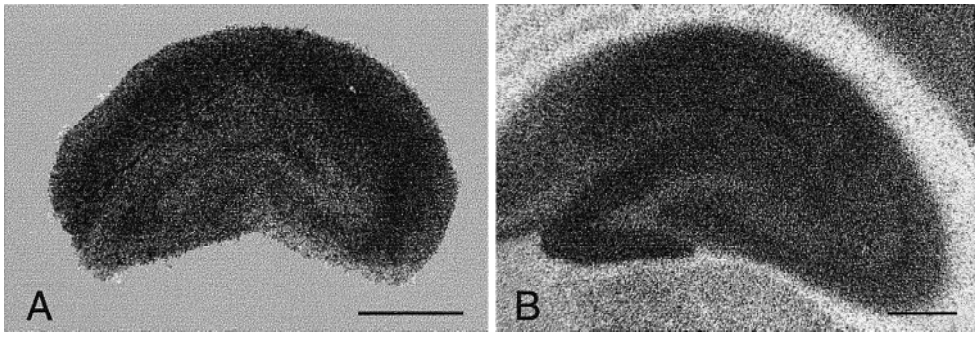

Fig. 4. ^{3}H-AMPA ligand binding in 4 week old hippocampal slice culture (**A**) and 5 week old hippoocampus *in vivo* (**B**), disclosing a near normal appearance in CA3 and CA1, but reduction in the dentate molecular layer in the slice culture. Scale bar: 0.5 mm. *Method*: Unfixed cultures and rat brains were frozen and cryostat sectioned at 20 μm, and the sections thaw-mounted on poly-L-lysine coated glass slides for storage at – 80°C until use. For binding the thawed sections were preincubated for 10 min in a solution of 50 mM Tris-HCl buffer (pH 7.1), 2.5 mM $CaCl_2$ and 100 mM KSCN at 20°C and then incubated at the same temperature for 40 min with 20 nM ^{3}H-AMPA (specific activity = 53.0 Ci/mmol) (Amersham, Denmark) in the same buffer. All slides with sections were subsequently rinsed for 3 × 1 sec in cold buffer and dipped for 1 sec in 15% glutaraldehyde/acetone. Adjacent sections were handled identically, but incubated in 1 mM ^{3}H-AMPA to define non-specific binding. Following incubation and rinses the slides with the sections were rapidly dried in cold air and exposed to ^{3}H-hyperfilm (Amersham, Denmark) in closed film cassettes at 40°C. After exposure for 8 weeks the films were developed in a Dektol solution (Kodak), diluted 1 : 9 with water

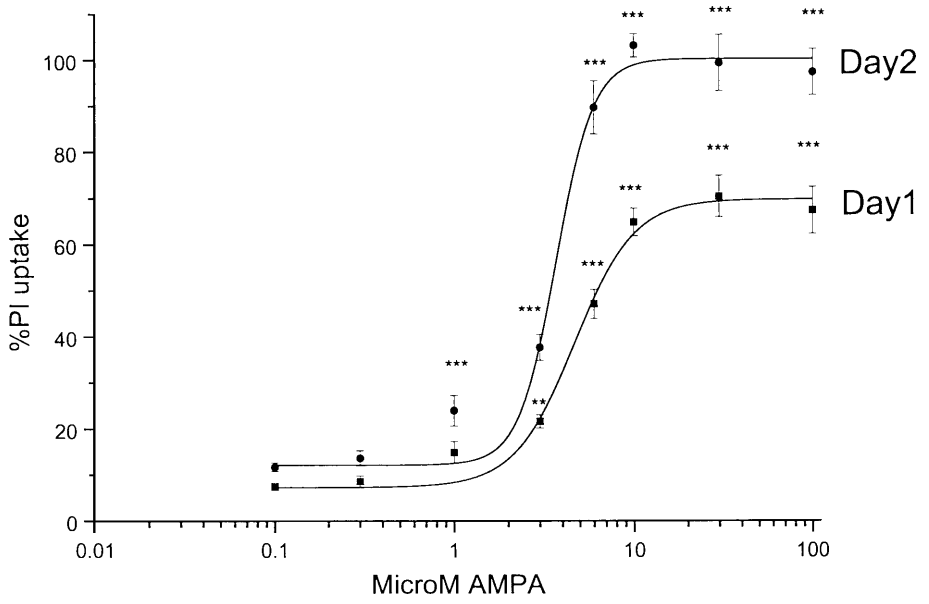

Fig. 5. Dose-response relationship between applied doses of AMPA and PI uptake in hippocampus, recorded 1 and 2 days after start of continuous exposure of hippocampal slice cultures to 0.1–100μM AMPA. For 2 days of exposure we found EC_{50} = *3.7μM AMPA*. The PI uptake is calculated as percentage of maximal PI uptake after exposure to the group of highest AMPA doses used (10–100μM). Data are shown as means +/− S.E.M., with n = 8–18. **P < 0.01, ***P < 0.001, ANOVA with Bonferoni correction, as compared to control for the different groups of cultures treated with AMPA

to 10μM NMDA (N-methyl-D-aspartate) for 48 hours thus displayed a significantly higher PI uptake in the CA1 pyramidal cell layer compared to the dentate granule cell and CA3 pyramidal cell layers after both the first and the second day of exposure (Fig. 6). The NMDA-induced degeneration in CA1 moreover occured faster, reaching a plateau within one day, while the degeneration of dentate granule cells still evolved during the second day, and possibly even later (not examined).

Current use of PI and other markers in corticostriatal slice cultures

PI uptake has also been used to monitor the excitotoxic effects of increasing doses of the glutamate agonists kainic acid (KA) and AMPA in corticostriatal slice cultures (Kristensen et al., 1999) (Figs. 7 and 8). Also the corticostriatal slice cultures showed good correlations between the density and distribution of the PI uptake and the Fluoro-Jade staining of cortex and striatum, LDH release to the medium, a general cell loss observed in Nissl staining, and loss of GABA'ergic cells, demonstrated by loss of γ-aminobutyric acid (GABA) immunocytochemical staining and loss of biochemically detected glutamic acid decarboxylase (GAD) activity from the cultures (Kristensen et al., 1999).

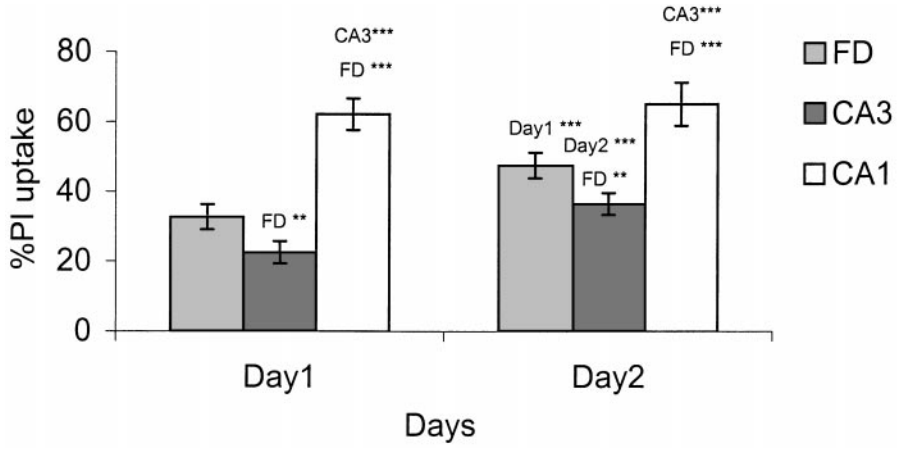

Fig. 6. PI uptake showing the characteristic CA1 lesion after one day and two days of exposure to 10 μM NMDA. Data are shown as means +/− S.E.M., with n = 10. **P < 0.01, ***P < 0.001, ANOVA for comparison of the different subfields, paired t-test for comparisons between first and second day of exposure. Note the higher and faster occuring PI uptake in CA1 with maximal level attained already after one day, while the lower PI uptake in fascia dentata dentate granule cells still evolved during the second day

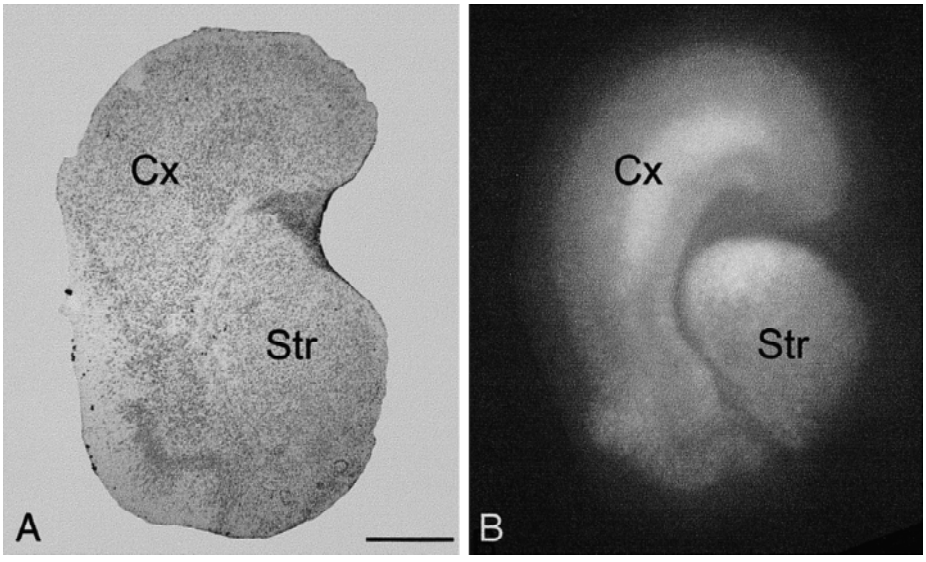

Fig. 7. Corticostriatal slice cultures grown for 4 weeks. **A** Toluidine blue cell staining of cryostat section of culture, illustrating preservation of regional cytoarchitectures in cortex (*Cx*) and striatum (*Str*). **B** Flourescence micrograph of PI uptake in culture after exposure to 24 μM KA for 48 h. PI-labelled, dead or dying neurons are present in both striatum (*Str*) and cortex (*Cx*). Scale bar: 1 mm

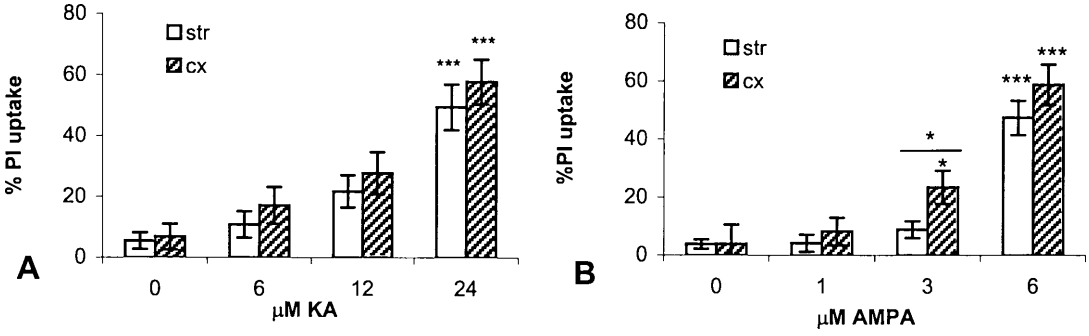

Fig. 8. Comparison of excitotoxic effects of KA and AMPA in striatal (*str*) and cortical (*cx*) parts of corticostriatal slice cultures, monitored by propidium iodide (*PI*) uptake. The PI uptake was recorded densitometrically 48 h after start of exposure to KA (**A**) or AMPA (**B**). The only significant difference in the vulnerability of the striatum and cortex was found after exposure to 3 μM AMPA. *P $<$ 0.05, t test for comparison of striatum and cortex; *** $P < 0.001$, ANOVA with Bonferoni correction for comparison with control

Different protective effects of NBQX against KA and AMPA in cortex and striatum

Although there was no clearcut differences in the susceptibility of the cortical and striatal parts of the corticostriatal slice co-cultures to KA and AMPA (Fig. 8), we observed a clear differential effect in the two brain areas, when the glutamate receptor antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline) was added at different concentrations together with 24 μM KA or 6 μM AMPA (Kristensen et al., 1999). Figure 9A illustrates how a low dose of 0.3 μM NBQX applied together with 24 μM KA significantly reduced the cortical PI uptake, and hence protected cortical neurons, without

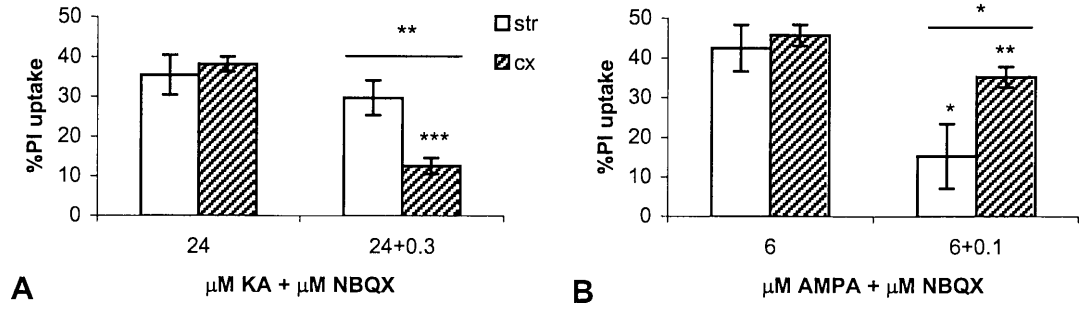

Fig. 9. Differential neuroprotective effects of NBQX on cortex and striatum, demonstrated by comparison of the striatal (*str*) and cortical (*cx*) uptake of propidium iodide (*PI*) in corticostriatal slice cultures after 48 h exposure to (**A**) 24 μM KA plus 0.3 μM NBQX and (**B**) 6 μM AMPA plus 0.1 μM NBQX. A low dose of NBQX (0.3 μM) protected better against KA in the cortex than in the striatum, while an even lower dose (0.1 μM) protected better against AMPA in the striatum than in the cortex. Data are shown as means +/− S.E.M., with n = 9 − 18. *P $<$ 0.05, **P $<$ 0.01, ***P $<$ 0.001, t-test for comparison of the KA and AMPA exposed groups and for comparison of striatum and cortex

having a similar effect on striatum and striatal neurons. When a slightly lower dose of 0.1 μM NBQX was applied together with 6 μM AMPA, the opposite occurred, namely a significant protection of striatal, but not cortical neurons (Fig. 9B). The protective effects of NBQX against KA and AMPA in striatum and cortex are in accordance with *in vivo* results, where injections of 25 nmol NBQX into the rat stiatum together with 10 nmol KA or 40 nmol AMPA have been shown to abolish the toxic effects of KA and AMPA (Massieu and Tapia, 1994). Addition of 10 μM quinoxalindiones to 300 μM KA and 500 μM AMPA in primary, cortical cell cultures also abolished the excititoxic effects of KA and AMPA (Frandsen et al., 1989; Jensen et al., 1998), but it should be noted that both *in vivo* and in dispersed cell cultures the applied doses of KA, AMPA and NBQX are much higher than used in the slice cultures. Besides its action on KA receptored, KA is also a weak AMPA receptor agonist, while the antagonist activity of NBQX is strongest against AMPA receptors. One explanation of why higher concentrations of NBQX were needed for the protection of the striatum than for protection of the cortex against KA is therefore that KA excerted more of its toxicity in the striatum directly via KA receptors. Again the results were in accordance with *in vivo* observations, where more NBQX was needed for striatal protection, if co-injected with KA compared to AMPA (Massieu and Tapia, 1994). Regarding the interpretation of the AMPA-induced effects and the interaction with NBQX in the corticostriatal slice cultures, the results suggest that AMPA receptors in the two regions are activated by AMPA with different potency or alternatively that NBQX acts with different potency on AMPA receptors in the two brain areas.

Modulatory effects of long-term exposure to kainic acid and NBQX

Besides or coupled to its designated role as information processing excitatory neurotransmitter in the central nervous system (CNS), glutamate is involved in developmental and adult synaptic plasticity and neurogenesis (McDonald and Johnston, 1990) and neurodegeneration (Dawson et al., 1995). Knowledge about possibilities to modulate the neuronal susceptibility to glutamate receptor activation and the expression and composition of glutamate receptors in the developing and the adult CNS is accordingly important, both for our understanding of normal brain functions and for the development of rational strategies for treatment of neurodegenerative diseases.

As a starting point we have exposed developing rat hippocampal slice cultures to low doses of KA or the AMPA/KA antagonist NBQX, with the purpose of studying inducible changes in KA excitotoxicity and corresponding changes in KA receptor subunit mRNA expression (Jakobsen et al., 2000).

For the long-term treatments hippocampal slice cultures, grown by the Roller tube method *ad modum* Gähwiler (1984) or the insert method *ad modum* Stoppini et al. (1991), were exposed to 2 μM KA or 0.3 μM NBQX (gift from NOVO Nordic, A/S) in serum-free medium from the 4th day after

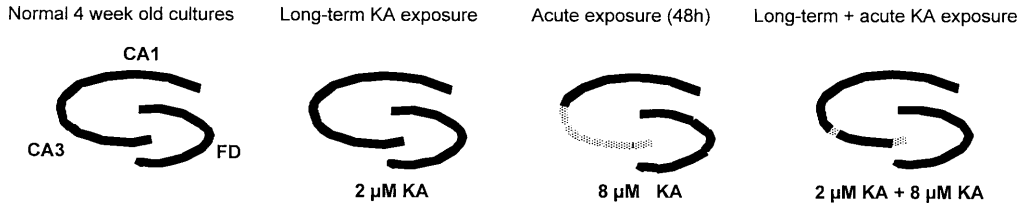

Fig. 10. Schematic illustration of the reduction in acute KA toxicity after treatment of hippocampal slice cultures from day 4 to day 25 *in vitro* by a non-toxic dose of 2μM KA. Acute exposure of control cultures to 8μM KA for 48 hrs followed by another 48 hrs in normal medium results in total loss of Nissl staining in CA3, but only minimal loss in the long-term treated cultures. Dotted parts represent cell layers with degeneration in terms of loss of Nissl staining

explantation, with medium change twice a week. After 3 weeks of treatment the long-term KA treated cultures were exposed to 8μM KA, the long-term NBQX treated cultures exposed to 3 or 5μM KA, and untreated, control cultures exposed to 3, 5 or 8μM KA for 48 hours, returned to normal medium for another 48 hrs, and then processed histologically. Other long-term treated cultures were tested for PI uptake both during the long-term exposure period and in relation to the acute KA exposure, while others were processed for *in situ* hybridization, using ^{35}S-labelled oligonucleotide probes for detection and densitometric quantitation of the mRNAs for the kainate receptor subunits GluR6, KA1 and KA2, as well as the AMPA receptor subunit GluR1.

The results of long-term, low dose KA treatment, with reduced susceptibility of the CA3 pyramidal cells to a subsequent high and normally toxic dose of KA, are shown schematically in Fig. 10. Reduced susceptibility of the CA3 pyramidal cells have also been found after brief exposures to subtoxic levels of KA with induction of heat shock protein (Best et al., 1996). We have not detected expression of heat shock protein in relation to our long-term, low dose exposure with KA, and therefore favour other explanations in our experimental conditions. One such explanation could be a change in KA receptor expression. Not having access to antibodies against the different KA1, KA2 and GluR5-7 proteins, we investigated possible changes at the mRNA levels by quantitative, autoradiographic *in situ* hybridization. The induced changes with downregulation of GluR6 and KA2 (and possibly KA1) mRNAs (Fig. 11A) are in accordance with our hypothesis of reduced expression of KA receptors after long-term, low dose (2μM) KA treatment of the cultures. In support of this modulability we found an oppositely directed effect with increased expression of KA1 and KA2 mRNAs in CA3 (and fascia dentata) in the cultures long-term exposed to 10μM NBQX (Fig. 11B). Other findings (not illustrated here) of a decreased expression of the AMPA receptor subunit protein GluR1, determined by Western blot, but an unchanged expression of GluR1 mRNA in the long-term KA treated cultures, remain so far unexplained, but may be related to the known cross-over action of KA on AMPA receptors.

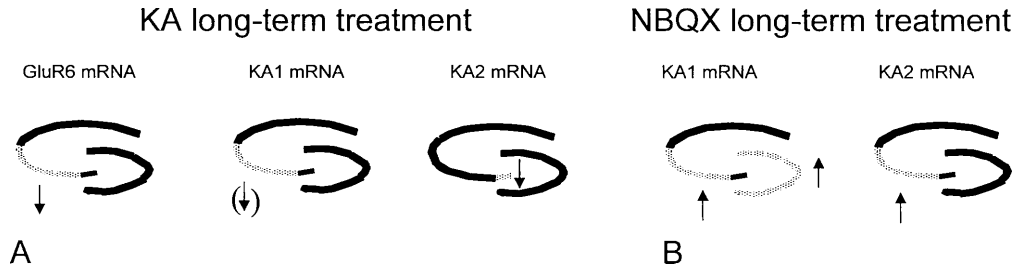

Fig. 11. **A** Changes in GluR6 and KA2 (and possibly KA1) receptor subunit mRNAs in hippocampal slice cultures long-term exposed to 2μM KA. **B** Changes in KA1 and KA2 receptor subunit mRNAs in hippocampal slice cultures long-term exposed to 0.3μM NBQX. Dotted areas show location of changes

Concluding this final part of the review, our observations have demonstrated that the expression of glutamate receptor subunit proteins and mRNAs are modifiable and that we can expect interactions between the different glutamate receptor types.

Conclusion

Brain slice cultures do, with predictable modifications relating to the isolation from the rest of the brain at explantation, remain organotypically organized. By choosing among the different culture techniques available, the slice cultures are easily accessible for most experimental manipulations, including choice of donor animal and *ex vivo* gene transfection provided that the explanted cells are still developing. Given the fact that the cultured slices in addition are easily accessible for analysis by a variety of techniques from molecular biology to functional imaging and electrophysiology, we can only recommend the continued use of brain slice cultures both for screening and mechanistic studies of neuroactive and neurotoxic compounds and for studies of receptor modulation and plasticity.

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References

Bahr BA (1995) Long-term hippocampal slices: a model system for investigating synaptic mechanisms and pathological processes. J Neurosci Res 42: 294–305

Barth A, Newell DW, Nguyen LB, Winn HR, Wender R, Meno JR, Janigro D (1997) Neurotoxicity in organotypic hippocampal slices mediated by adenosine analogues and nitric oxide. Brain Res 762: 79–88

Bergold PJ, Casaccia Bonnefil P (1997) Preparation of organotypic hippocampal slice cultures using the membrane filter method. Methods Mol Biol 72: 15–22

Best N, Sundström LE, Mitchell J, Wheal HV (1996) Pre-exposure to subtoxic levels prevents kainic acid lesions in organotypic hippocampal slice cultures: effects of kainic acid on parvalbumin-immunoreactive neurons and expression of heat shock protein 72 following the induction of tolerance. Eur J Neurosci 8: 1209–1219

Bevensee MO, Schwiening CJ, Boron WF (1995) Use of BCECF and propidium iodide to assess membrane integrity of acutely isolated CA1 neurons from rat hippocampus. J Neurosci Meth 58: 61–75

Bruce AJ, Malfroy B, Baudry M (1996) Beta-amyloid toxicity in organotypic hippocampal cultures: Protection by EUK-8, a synthetic catalytic free radical scavenger. Proc Natl Acad Sci USA 93: 2312–2316

Dahl-Jørgensen A, Østergaard K, Pedersen EB, Zimmer J (1999) Serum and CNTF stimulate oligodendroglia and reduce fiber ourgrowth from striatal cultures. Exp Neurol 157: 88–95

Frandsen A, Drejer J, Schousboe A (1989) Direct evidence that excitotoxicity in cultured neurons is mediated via N-methyl-D-aspartate (NMDA) as well as non-NMDA receptors. J Neurochem 53: 297–299

Frotscher M, Heimrich B, Schwegler H (1990) Plasticity of identified neurons in slice cultures of hippocampus: a combined Golgi/electron microscopic and immunocytochemical study. Progr Brain Res 83: 323–339

Gähwiler BH (1984) Slice cultures of cerebellar, hippocampal and hypothalamic tissue. Experientia 40: 235–243

Gähwiler BH (1988) Organotypic cultures of neural tissue. Trends Neurosci 11: 484–489

Gähwiler BH, Capogna M, Debanne D, McKinney JS, Thompson SM (1997) Organotypic slice cultures: a technique has come of age. Trends Neurosci 20: 471–477

Hsu SS, Newell DW, Tucker A, Malouf AT, Winn HR (1994) Adenosinergic modulation of CA1 neuronal tolerance to glucose deprivation in organotypic hippocampal cultures. Neurosci Lett 178: 189–192

Jakobsen B, Kristensen BW, Diemer NH, Zimmer J (2000) Modulation of kainic acid excitotoxicity and glutamate receptor subunit mRNA expression in hippocampal slice cultures. Exp Neurol (submitted)

Jahnsen H, Kristensen BW, Thiébaud P, Noraberg J, Jacobsen B, Bove M, Martinoia S, Koudelka-Hep M, Grattarola M, Zimmer J (1999) Coupling of organotypic brain slice cultures to silicon-based arrays of electrodes. Meth Enzymol 18: 160–172

Jensen JB, Pickering DS, Schousboe A (1998) AMPA receptor mediated excitotoxicity in neocortical neurons is developmentally regulated and dependent upon receptor desensitization. Neurochem Int 32: 505–513

Koh JY, Choi DW (1987) Quantitative determination of glutamate mediated cortical neuronal injury in cell culture by lactate dehydrogenase efflux assay. J Neurosci Meth 20: 83–90

Kristensen BW, Noraberg J, Jakobsen B, Gramsbergen JB, Ebert B, Zimmer J (1999) Excitotoxic effects of non-NMDA receptor agonists in organotypic corticostriatal slice cultures. Brain Res 841: 143–159

Lizard G, Fournel S, Genestier L, Dhedin N, Chaput C, Flacher M, Mutin M, Panaye G, Revillard JP (1995) Kinetics of plasma membrane and mitochondrial alterations in cells undergoing apoptosis. Cytometry 21: 275–283

Macklis JD, Madison RD (1990) Progressive incorporation of propidium iodide in cultured mouse neurons correlates with declining electrophysiological status: a fluorescence scale of membrane integrity. J Neurosci Meth 31: 43–46

Massieu L, Tapia R (1994) 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(f) quinoxaline protects against both AMPA and kainate-induced lesions in rat striatum in vivo. Neuroscience 59: 931–938

Meyer M, Zimmer J, Seiler RW, Widmer HR (1999) GDNF increases the density of cells containing calbindin but not of cells containing calretinin in cultured rat and human fetal nigral tissue. Cell Transpl 8: 25–36

Nadler JV, Perry BW, Gentry C, Cotman CW (1980) Degeneration of hippocampal CA3 pyramidal cells induced by intraventricular kainic acid. J Comp Neurol 192: 333–359

Nakagami Y, Saito H, Matsuki N (1997) Basic fibroblast growth factor and brain-derived neurotrophic factor promote survival and neuronal circuit formation in organotypic hippocampal culture. Jpn J Pharmacol 75: 319–326

Newell DW, Barth A, Papermaster V, Malouf AT (1995) Glutamate and non-glutamate receptor mediated toxicity caused by oxygen and glucose deprivation in organotypic hippocampal cultures. J Neurosci 15: 7702–7711

Newell DW, Hsu SS, Papermaster V, Malouf AT (1993) Colchicine is selectively neurotoxic to dentate granule cells in organotypic cultures of rat hippocampus. Neurotoxicology 14: 375–380

Noraberg J, Zimmer J (1998) Ethanol induces MAP2 changes in organotypic hippocampal slice cultures. NeuroReport 9: 3177–3182

Noraberg J, Gramsbergen JBP, Fonnum F, Zimmer J (1997) Neurotoxicity of trimethyltin (TMT), ethanol and ketones in hippocampal slice cultures. Soc Neurosci Abstracts 23: 1935

Noraberg J, Gramsbergen JBG, Fonnum F, Zimmer J (1998) Trimethyltin (TMT) neurotoxicity in organotypic rat hippocampal cultures. Brain Res 783: 305–315

Noraberg J, Kristensen BW, Zimmer J (1999) Markers for neuronal degeneration in organotypic slice cultures. Brain Res Protocols 3: 278–290

Østergaard K (1993) Organotypic slice cultures of the rat striatum – I. A histochemical and immunocytochemical study of acetylcholinesterase, choline acetyltransferase, glutamate decarboxylase and GABA. Neuroscience 53: 679–693

Østergaard K, Schou JP, Zimmer J (1990) Rat ventral mesencephalon grown as organotypic slice cultures and co-cultured with striatum, hippocampus, and cerebellum. Exp Brain Res 82: 547–565

Østergaard K, Schou JP, Gähwiler BH, Zimmer J (1991) Tyrosine hydroxylase immunoreactive neurons in organotypic slice cultures of the rat striatum and neocortex. Exp Brain Res 83: 357–365

Østergaard K, Finsen B, Zimmer J (1995) Organotypic slice cultures of the rat striatum: an immunocytochemical, histochemical and in situ hybridization study of somatostatin, neuropeptide Y, nicotinamide adenine dinucleotide phosphate-diaphorase, and enkephalin. Exp Brain Res 103: 70–84

Østergaard K, Jones SA, Hyman C, Zimmer J (1996) Effects of donor age and brain-derived neurotrophic factor on the survival of dopaminergic neurons and axonal growth in postnatal rat nigrostriatal cocultures. Exp Neurol 142: 340–350

Plenz D, Aertsen A (1996a) Neural dynamics in cortex-striatum co-cultures I. Anatomy and electrophysiology of neuronal cell types. Neuroscience 70: 861–891

Plenz D, Aertsen A (1996b) Neural dynamics in cortex-striatum co-cultures II. Spatiotemporal characteristics of neuronal activity. Neuroscience 70: 893–924

Plenz D, Kitai ST (1996) Organotypic cortex-striatum-mesencephalon cultures: the nigrostriatal pathway. Neurosci Lett 209: 177–180

Plenz D, Kitai ST (1998) Up and down states in striatal medium spiny neurons simultaneously recorded with spontaneous activity in fast-spiking interneurons studied in cortex-striatum-substantia nigra organotypic cultures. J Neurosci 18: 266–283

Pozzo Miller LD, Mahanty NK, Connor JA, Landis DM (1994) Spontaneous pyramidal cell death in organotypic slice cultures from rat hippocampus is prevented by glutamate receptor antagonists. Neuroscience 63: 471–487

Pringle AK, Sundstrom LE, Wilde GJ, Williams LR, Iannotti F (1996) Brain-derived neurotrophic factor, but not neurotrophin-3, prevents ischaemia-induced neuronal cell death in organotypic rat hippocampal slice cultures. Neurosci Lett 211: 203–206

Pringle AK, Angunawela R, Wilde GJ, Mepham JA, Sundstrom LE, Iannotti F (1997a) Induction of 72 kDa heat-shock protein following sub-lethal oxygen deprivation in organotypic hippocampal slice cultures. Neuropathol Appl Neurobiol 23: 289–298

Pringle AK, Iannotti F, Sundstrom LE (1997b) Neuroprotection by both NMDA and non-NMDA receptor antagonists in in vitro ischemia. Brain Res 755: 36–46

Rimvall K, Keller F, Waser PG (1987) Selective kainic acid lesions in cultured explants of rat hippocampus. Acta Neuropathol Berl 74: 183–190

Romijn HJ, Ruijter JM, Wolters PS (1988) Hypoxia preferentially destroys GABAergic neurons in developing rat neocortex explants in culture. Exp Neurol 100: 332–340

Sakaguchi T, Okada M, Kuno M, Kawasaki K (1997) Dual mode of N-methyl-D-aspartate-induced neuronal death in hippocampal slice cultures in relation to N-methyl-D-aspartate receptor properties. Neuroscience 76: 411–423

Sautter J, Meyer M, Spenger C, Seiler RW, Widmer HR (1998) Effects of combined BDNF and GDNF treatment on cultured dopaminergic midbrain neurons. NeuroReport 9: 1093–1096

Schmued LC, Albertson C, Slikker W Jr (1997) Fluoro-Jade: a novel fluorochrome for the sensitive and reliable histochemical localization of neuronal degeneration. Brain Res 751: 37–46

Schousboe I, Tønder N, Zimmer J, Schousboe A (1993) A developmental study of lactate dehydrogenase isoenzyme and aspartate aminotransferase activity in organotypic rat hippocampal slice cultures and primary cultures of mouse neocortical and cerebellar neurons. Int J Develop Neurosci 11: 765–772

Stoppini L, Buchs PA, Muller D (1991) A simple method for organotypic cultures of nervous tissue. J Neurosci Meth 37: 173–182

Tasker RC, Coyle JT, Vornov JJ (1992) The regional vulnerability to hypoglycemia-induced neurotoxicity in organotypic hippocampal culture: protection by early tetrodotoxin or delayed MK-801. J Neurosci 12: 4298–4308

Thiebaud P, Rooij NF de, Koudelka Hep M, Stoppini L (1997) Microelectrode arrays for electrophysiological monitoring of hippocampal organotypic slice cultures, IEEE Trans. Biomed Eng 44: 1159–1163

Thomas M, Davis MI, Monaghan DT, Morrisett RA (1998) Organotypic brain slice cultures for functional analysis of alcohol-related disordes: novel versus conventional preparations. Alcohol Clin Exp Res 22: 51–59

Tønder N, Zimmer J, Goller H-J, Wree A (1993) Area specific functional activity in organotypic hippocampal slicecultures. A 2-deoxyglucose study. J Hirnforsch 34: 109–114

Torp R, Haug FM, Tønder N, Zimmer J, Ottersen OP (1992) Neuroactive amino acids in organotypic slice cultures of the rat hippocampus: an immunocytochemical study of the distribution of GABA, glutamate, glutamine and taurine. Neuroscience 46: 807–843

Vassault A (1983) Lactate dehydrogenase. UV-method with pyruvate and NADH. In: Bergmeyer HU, Bergmeyer J, Grossl M (eds) Methods of enzymatic analysis, 3rd edn. Verlag Chemie GmbH, Weinheim, pp 118–126

Vitale M, Zamai L, Mazzotti G, Cataldi A, Falcieri E (1993) Differential kinetics of propidium iodide uptake in apoptotic and necrotic thymocytes. Histochemistry 100: 223–229

Vornov JJ, Tasker RC, Coyle JT (1991) Direct observation of the agonist-specific regional vulnerability to glutamate, NMDA, and kainate neurotoxicity in organotypic hippocampal cultures. Exp Neurol 114: 11–22

Vornov JJ, Tasker RC, Coyle JT (1994) Delayed protection by MK-801 and tetrodotoxin in a rat organotypic hippocampal culture model of ischemia. Stroke 25: 457–464

Vornov JJ, Park J, Thomas AG (1998) Regional vulnerability to endogenous and exogenous oxidative stress in organotypic hippocampal culture. Exp Neurol 149: 109–122

Whetsell WO Jr, Schwarcz R (1983) The organotypic tissue culture model of corticostriatal system used for examining amino acid neurotoxicity and its antagonism: studies on kainic acid, quinolinic acid and (-)2-amino-7-phosphonoheptanoic acid. J Neural Transm [Suppl 19]: 53–63

Zimmer J, Gähwiler BG (1984) Cellular and connective organization of slice cultures of the rat hippocampus and fascia dentata. J Comp Neurol 228: 432–446

Zimmer J, Gähwiler BH (1987) Growth of hippocampal mossy fibers: a lesion and coculture study of organotypic slice cultures. J Comp Neurol 264: 1–13

Authors' address: Prof. Jens Zimmer, Department of Anatomy & Neurobiology, SDU-University of Odense, Winsløwparken 21, DK-5000 Odense C, Denmark,
Fax +45 6590 6321, E-mail: zimmer@imbmed.sdu.dk

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