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A Bridge Between *in vitro* and *in vivo* Studies in Neuroscience: Organotypic Brain Slice Cultures

Nörobilimde *in vitro* ve *in vivo* Çalışmalar Arasında Bir Köprü: Beyin Organotipik Kesit Kültürleri

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ABSTRACT

In vitro and *in vivo* models are efficiently used systems in neuroscience research to study the brain in normal or pathological conditions. There are many advantages to these systems, yet they also have significant limitations. *In vitro* cell cultures offer the opportunity to investigate the cell basics or primary response of a cell population against any treatment. However, these models do not always predict *in vivo* behavior. *In vivo* animal studies constitute the most realistic platform for research and therapeutic approaches, yet they are laborious, open to secondary complications and painful or stressful for the animals from an ethical point of view. Organotypic brain slice cultures provide an *in vivo*-like environment since they maintain three-dimensional cytoarchitecture of the brain thus enable to study many cell types in one system and allow precise control of the microenvironment. In this review, we will focus on the history and key features of organotypic brain slice cultures as well as its preparation.

Keywords: Organotypic brain slice culture, whole-brain slice culture, membrane interface method

ÖZET

In vitro ve *in vivo* modeller, normal ve patolojik koşullarda beyni incelemek üzere nörobilim araştırmalarında etkili bir şekilde kullanılan sistemlerdir. Bu sistemlerin çok sayıda avantajı bulunmasına karşın aynı zamanda kısıtlı kaldıkları önemli noktalar da vardır. *In vitro* hücre kültürleri, hücrenin temellerini veya herhangi bir uygulamaya karşı bir hücre popülasyonunun birincil yanıtını incelemeye olanak tanırlar. Ancak, bu modeller her zaman *in vivo* davranışı yansıtmazlar. *In vivo* hayvan çalışmaları, araştırma ve terapötik yaklaşımlar için en gerçekçi platformları oluşturmalarına rağmen son derece zahmetli, ikincil komplikasyonlara açık ve etik açıdan hayvanlar için ıstıraplı ve stresli olan sistemlerdir. Beyin organotipik kesit kültürleri, beynin üç-boyutlu hücre mimarisini korudukları için *in vivo*-benzeri bir ortam sağlar, böylelikle tek bir sistemde çok sayıda hücre tipinin incelenmesine ve mikroçevrenin titiz bir şekilde kontrolüne imkan sağlar. Bu derlemede, beyin organotipik kesit kültürlerinin tarihi ve anahtar özellikleriyle birlikte hazırlanışına da değineceğiz.

Anahtar Kelimeler: Beyin organotipik kesit kültürleri, tüm beyin kesit kültürü, membran arayüz yöntemi

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INTRODUCTION

In vitro cell cultures are one of the most widely used model systems in cellular and molecular biology since it allows to investigate the biology, biochemistry, physiology and metabolism of the cells in health and disease conditions. However, such cellular systems do not mirror the actual nature of an *in vivo* environment since isolated cells are lack of interactions with other cell types and extracellular matrix (1). Over the last decades, organotypic cultures were developed to overcome this disadvantage of *in vitro* cell cultures (2). Organotypic cultures are efficient models since they preserve the cytoarchitecture and the microenvironment of the tissue (3).

It is important to evaluate all findings from single neuron experiments to animal studies together due to the complexity of the brain (4). *In vitro* cell culture models of the neuron, astrocyte, microglia or neuron-like cell lines such as SH-SY5Y human neuroblastoma cells, rat pheochromocytoma cells (PC12) and human embryonic kidney 293 (HEK 293) cells let us understand the molecular and physiological basics of the brain cells and how these cells respond individually to any condition. The findings obtained from *in vitro* studies must be proven by *in vivo* models since the cells may act differently in their natural microenvironment. Even though *in vivo* animal models are the best systems for any kind of research area,

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they have significant limitations due to weak control of chemical and physical complications. At this point, organotypic brain slice cultures serve as a bridge and fill the gap between *in vitro* and *in vivo*.

Organotypic brain slice cultures are convenient and powerful models for almost all areas of neuroscience studies since these cultures maintain the three-dimensional architecture of the brain, neuronal networks and synaptic organization, and contain nearly all brain cells such as neurons, astrocytes and microglia. Therefore, these cultures enable neuroscientists to study many cell types simultaneously in an *in vivo*-like environment. In this review, we will summarize organotypic brain slice cultures in terms of its historical development, preparation, advantages and disadvantages over *in vitro* and *in vivo* models as well as factors that are important for the culture.

ORGANOTYPIC BRAIN SLICE CULTURES: THE HISTORY OF ITS DEVELOPMENT

The basis of organotypic slice culture is based on explant tissue culture experiments. As excellently reviewed by Crain, the term "organotypic" was introduced for the first time by Maximov in 1925 in order to describe cultured tissues with more organized growth and differentiation. Then in 1951, Fell described organotypic as "the tissue largely retains its characteristic architecture, remains functional and if derived from undifferentiated material, it may develop in culture in a surprisingly normal way" (5). The first examples of tissue culture experiments referred as "organotypic culture" were seen in the studies on development and differentiation of several organs of chick embryo such as eye (6), thyroid gland (7), lung (8) and intestine (9).

The initial cultivation of a central nervous system (CNS) tissue was performed by Harrison in 1907 with spinal cord of frog embryo (10). Later in 1913, Ingebrigtsen cultured brain tissues of the chick embryo, rabbit, cat and dog (11). Over the years, many scientists have sought a way to maintain the brain tissue alive and intact for a long period *in vitro*, thus developed several techniques. Among these techniques, the roller-tube and membrane interface techniques are the most widely used.

The roller-tube technique for brain tissue was first introduced by Hogue (12) and then modified by many scientists in years. The greatest improvement for this technique was done by Gähwiler who used thinner brain slices (300-400 µm thick) which are more suitable for physiological and pharmacological studies (13). In this technique each slice is attached on a glass coverslip either by a plasma clot or a collagen matrix. Each coverslip is singly inserted in a tube that contains culture medium and then the tubes are placed in a roller drum located in a humidified incubator at 37°C. Roller drum provides a slow rotation which makes these slices constantly oxygenated to ensure their survival. The slices cultured with the roller-tube technique reach a thickness of approximately 50 µm (almost a monolayer thick) after a few weeks *in vitro* which makes it possible to access individual cells. (14). There are some disadvantages of this method: falling-off of some of the slices during cultivation and the possibility of synaptic reorganization and sprouting due to embedding the tissue in clot and the static conditions.

To overcome these limitations, in 1991 Stoppini et.al. described a different and easier method for organotypic slice cultures in which tissue is placed on a semi-porous membrane at the interface between air and culture medium (15). In this "membrane" or "interface" technique, membrane inserts which the slices are transferred on are placed into a petri dish containing a very small amount of culture medium below, and by that the tissue is directly exposed to the air for oxygenation. Semi-porous membrane allows diffusion of the substances in the culture medium that are required for slice survival. Compared to the roller-tube method, the tissue remains thicker with a thickness of 100-150 µm (a few layers of cells thick) in a few weeks *in vitro* depending on the initial thickness which makes them usable for both electrophysiological and analytical techniques. Moreover, this method is more suitable for any kind of application such as chemical or drug treatment and gene silencing or overexpressing since the slices are not covered with any material.

Both roller-tube and membrane techniques preserve the cytoarchitecture of the tissue yet the major differences between these two methods are the final thickness of the slice and the future analysis to be performed. Since its easier to prepare and have a few advantages over the roller-tube method, most of the laboratories prefer membrane technique currently, including ours.

CRUCIAL FACTORS TO CONSIDER FOR ORGANOTYPIC BRAIN SLICE CULTURES

Age of the Animal

As reviewed well by Humpel (2) and Croft et al. (16), the age of the donor animal is an important factor for tissue survival and cultivation time in organotypic brain slice cultures. Brains of animals at early postnatal days (usually P6-P8) are commonly used for slice cultures since they are more resistant to mechanical trauma that occurs during the slice preparation compared to the adult brains. Moreover, cytoarchitecture and synaptic connections are already established in most brain areas (17). Slice cultures may also be prepared from embryonic animals, yet they are not suitable well for long-term cultivation since the cells are still in their migratory phase and tissue organization usually deteriorates. Using early postnatal animals has another advantage: their brains are larger than embryonic ones, hereby it is easier to dissect (14).

Even though organotypic brain slice cultures are typically prepared from early postnatal animals, some researchers put in the effort to culture slices from adult animal brains (18-23) and human brain biopsies (24, 25) or even post-mortem human brains (26). However, preparing organotypic brain slice culture from adult brains is more challenging and requires to be optimized well for long-term culturing such as culturing at lower temperatures, optimizing culture medium components or reducing the thickness since the cell survival is very limited and cytoarchitectural organization of the tissue does not retain for a long time (16).

Brain Regions to be Cultured

Organotypic brain slice cultures can be successfully prepared from almost all brain regions including hippocampus (27), cerebellum (28), cortex (29), thalamus (30), hypothalamus (31), striatum (32) and substantia nigra (33). Yet, hippocampus and cerebellum are the most widely studied two brain regions.

The great majority of the studies in the literature performed slice culture from a specific brain region while some co-cultured two or more regions that take part in a pathway. On the other hand, whole coronal or sagittal slices can also be used to study desired target brain regions. With this type of culture, a more *in vivo*-like environment can be established. Moreover, it was reported that whole brain slices are more resistant because the neurons are less axotomized in that way (34). There are several examples for culturing of whole sagittal (3, 35-37) and coronal (20, 38-42) slices of the brain. Furthermore, some groups have reported successfully culturing of whole coronal brain slices of adult rat (42) and mouse (20).

However, obtaining whole-brain slices intact and keeping the slices alive for long-term cultivation is a more complex process and requires more expertise and some modifications of culture medium compositions such as the addition of exogenous growth factors in order to support neuronal survival. For example, Ullrich et. al. reported a method for whole sagittal

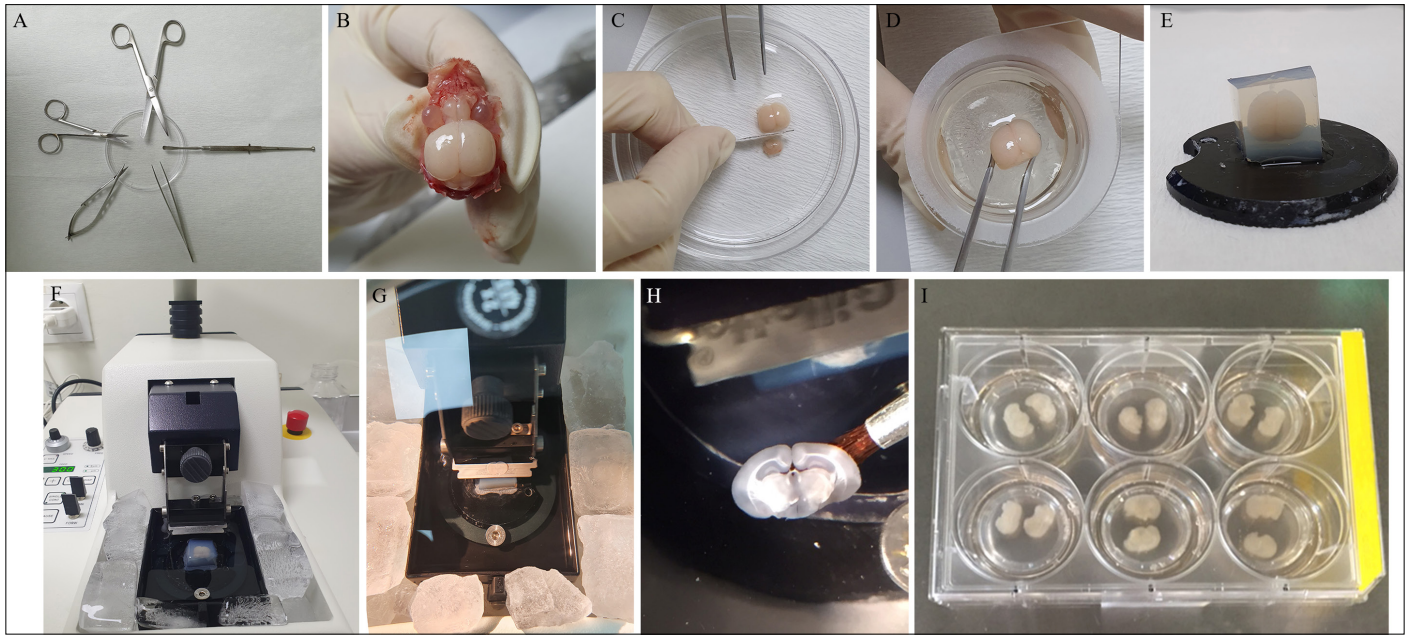

Figure 1: Preparation of organotypic whole-brain coronal slice culture. (A) Dissection tools are used for preparation. **(B)** The brain of the rat at P7 is carefully dissected under aseptic conditions. **(C)** The olfactory bulb and cerebellum are removed by a razor blade. **(D)** Trimmed brain is embedded into a low melting agarose. **(E)** Solid agarose is trimmed into a cube by a razor blade and glued to the specimen holder of the vibratome. **(F)** The specimen holder is placed into buffer tray that is filled with cold dissection medium. **(G)** The brain is coronally sliced into 100-400 µm thick sections by vibratome. **(H)** Coronal slices collected by a paintbrush and put into cold dissection medium. **(I)** Slides are transferred onto membrane inserts that are placed in a 6-well plate.

brain slice culture to study cholinergic and dopaminergic neurons together and pointed out that the addition of exogenous growth factors is necessary for neuronal survival (36). In any case, the culture conditions need to be determined experimentally for long-term culturing of whole sagittal or coronal brain slices depending on the brain region content of the slice that is desired to be cultured since each brain region have particular neuron types with different requirements. In our laboratory, we successfully culture whole coronal brain slices of neonatal (P7) rat brain containing hippocampus, cortex, striatum, thalamus and hypothalamus for 2 weeks.

Survival of the Slices

Keeping the slices alive for a long-term period is another challenging process as much as the preparation of the slices, since neurons are more sensitive compared to the other cell types. During the first week of the cultivation, healthy slices get thinner and splay and their color becomes transparent. These are the significant macroscopic indications of a healthy slice. Whitish-opaque remaining slices -whole slice or a part of it- should be considered as unhealthy and should be discarded from the study. Moreover, in a healthy slice, cell spreading out of the edges is seen during this period (2).

Even though these indications are the most distinct signs for slice vitality, in some circumstances, slices that appear macroscopically normal may not actually be healthy. Atrophy may occur in the slices in time, and this is most likely due to inefficient or inappropriate medium composition. Other than that, the age of the animal, the thickness of the slice, preparation conditions and speed are the major factors that affect slice survival.

PREPARATION OF ORGANOTYPIC SLICE CULTURES

It is important to note that preparing organotypic brain slice cultures necessitate a good knowledge of brain anatomy. Moreover, high technical skill is required for successful dissection of the brain especially from early postnatal animals (43). In this section, we will briefly mention the preparation of organotypic brain slice culture using membrane interface technique.

After decapitation of the animal, the brain is carefully dissected under aseptic conditions and put into a cold dissection medium composed of a balanced salt solution placed in ice. Optionally, the brain region to be cultured may also be dissected under a microscope after removal of the brain. The brain can be trimmed or split into two hemispheres by a razor blade. For whole-brain slices, the brain is embedded into a low melting agarose and then glued to the specimen holder of the vibratome. Buffer tray of the vibratome is filled with cold dissection medium and the surrounding platform is covered with ice. Keeping the brain cold is a crucial step for successful slicing and slice survival. Thereafter, the brain is sliced into 100-400 µm thick sections either sagittally or coronally. The slices collected in cold dissection medium by a paintbrush then are transferred onto semipermeable membrane inserts that are placed in a 6-well plate containing culture medium and cultured at 37°C and 5% CO_2 in a humidified chamber. The culture medium is changed twice a week. Generally, culture medium that is defined by Stoppini et al. (15) is used in membrane interface technique, yet as mentioned before its composition should modified experimentally depending on the brain region content of the slice that is desired to be cultured. The general workflow of this protocol is schematized in Figure 1.

Gliosis and microglial activation occur due to the endogenous release of enzymes and ions during slicing (4, 44). Hence, the slices should be cultured at least 10 days to minimize this activation before any kind of experiment to be performed. Figure 2 represents a two-week-old healthy whole-brain coronal slice and immunofluorescence labeling of the slices.

ADVANTAGES AND DISADVANTAGES

The major advantage of organotypic brain slice culture is that they preserve the cytoarchitecture of the brain and contain nearly all cell types thus allowing them to be studied in their microenvironment. These cultures maintain the morphological and physiological features of the brain such as cell-cell interactions, neuronal networks and synaptic organization similar to *in vivo* conditions (16, 17).

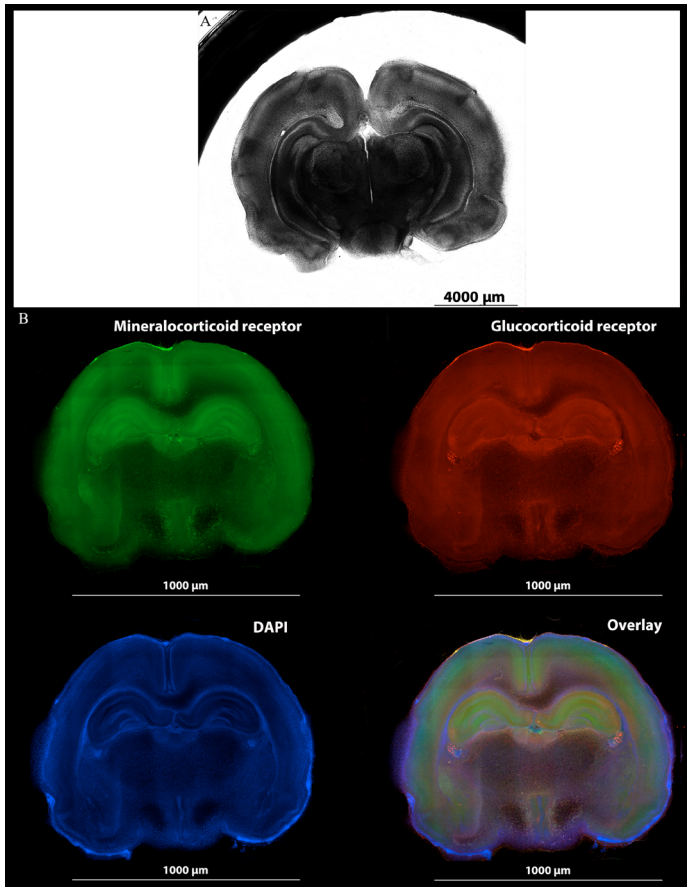

Figure 2: Whole-brain coronal slices. (A) A two-week-old slice under brightfield microscope. **(B)** Immunofluorescence labeling of the slice. All images were taken with BioTek Lionheart FX Automated Microscope (BioTek Instruments, Winooski, VT, USA) and Gen5 software was used to obtain stitched images. Magnification: 4X

Ethically, organotypic brain slice cultures offer a potentially alternative system to severe *in vivo* animal studies. *In vivo* animal experiments can cause significant pain or stress. Moreover, organotypic brain slice cultures reduce the number of animals required for an *in vivo* experiment since multiple slices can be obtained from one animal (16). Even though organotypic brain slice cultures are powerful models, they cannot substitute for *in vivo* models, since they do not represent the systemic or behavioral outcome.

These cultures are suitable for any kind of application ranging from drug, peptide or chemical treatments to gene alterations. Moreover, organotypic brain slice cultures offer researchers the opportunity to perform various analytical techniques such as immunofluorescent labeling, enzyme-linked immunosorbent assay (ELISA), western blot and quantitative real-time polymerase chain reaction (qRT-PCR) as well as electrophysiological recordings and stimulations. In summary, organotypic brain slice cultures are excellent model systems for almost all areas of neuroscience studies.

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REFERENCES

1. Segeritz CP, Vallier L. Cell Culture: Growing Cells as Model Systems In Vitro. In: Jalali M, Saldanha F, Jalali M, editors. Basic Science Methods for Clinical Researchers. 1st ed2017. p. 151-172.
2. Humpel C. Organotypic brain slice cultures: A review. Neuroscience. 2015;305:86-98. [Crossref]
3. Daviaud N, Garbayo E, Lautram N, Franconi F, Lemaire L, Perez-Pinzon M, Montero-Menei CN. Modeling nigrostriatal degeneration in organotypic cultures, a new ex vivo model of Parkinson's disease. Neuroscience. 2014;256:10-22. [Crossref]
4. Pena F. Organotypic cultures as tool to test long-term effects of chemicals on the nervous system. Curr Med Chem. 2010;17(10):987-1001. [Crossref]
5. Crain SM. Development of "organotypic" bioelectric activities in central nervous tissues during maturation in culture. Int Rev Neurobiol. 1966;9:1-43. [Crossref]
6. Reinbold R. [Organotypic differentiation of the eye of the chick embryo in vitro]. C R Seances Soc Biol Fil. 1954;148(15-18):1493-1495. [Crossref]
7. Gaillard PJ. Differentiation phenomena in explants of the embryonic chick thyroid gland. Acta Physiol Pharmacol Neerl. 1954;3(3):439-448. [Crossref]
8. Loffredo Sampaolo C, Sampaolo G. [Organotypic cultures of chick embryo lung; some histologic and histochemical aspects]. Boll Soc Ital Biol Sper. 1956;32(7-8):797-801. [Crossref]
9. Monesi V. Differentiation of argyrophil and argentaffin cells in organotypic cultures of embryonic chick intestine. J Embryol Exp Morphol. 1960;8:302-313. [Crossref]
10. Harrison RG. Observations on the living developing nerve fiber. Exp Biol Med. 1907;4(1):140-143. [Crossref]
11. Ingebrigtsen R. Studies of the Degeneration and Regeneration of Axis Cylinders in Vitro. J Exp Med. 1913;17(2):182-191. [Crossref]
12. Hogue MJ. Human fetal brain cells in tissue cultures; their identification and motility. J Exp Zool. 1947;106(1):85-107. [Crossref]
13. Gahwiler BH. Organotypic monolayer cultures of nervous tissue. J Neurosci Methods. 1981;4(4):329-342. [Crossref]
14. Gahwiler BH, Capogna M, Debanne D, McKinney RA, Thompson SM. Organotypic slice cultures: a technique has come of age. Trends Neurosci. 1997;20(10):471-477. [Crossref]
15. Stoppini L, Buchs PA, Muller D. A simple method for organotypic cultures of nervous tissue. J Neurosci Methods. 1991;37(2):173-182. [Crossref]
16. Croft CL, Futch HS, Moore BD, Golde TE. Organotypic brain slice cultures to model neurodegenerative proteinopathies. Mol Neurodegener. 2019;14(1):45. [Crossref]
17. Cho S, Wood A, Bowlby MR. Brain slices as models for neurodegenerative disease and screening platforms to identify novel therapeutics. Curr Neuropharmacol. 2007;5(1):19-33. [Crossref]
18. Su T, Paradiso B, Long YS, Liao WP, Simonato M. Evaluation of cell damage in organotypic hippocampal slice culture from adult mouse: a potential model system to study neuroprotection. Brain Res. 2011;1385:68-76. [Crossref]
19. Wilhelmi E, Schoder UH, Benabdallah A, Sieg F, Breder J, Reymann KG. Organotypic brain-slice cultures from adult rats: approaches for a prolonged culture time. Altern Lab Anim. 2002;30(3):275-283. [Crossref]
20. Staal JA, Alexander SR, Liu Y, Dickson TD, Vickers JC. Characterization of cortical neuronal and glial alterations during culture of organotypic whole brain slices from neonatal and mature mice. PLoS One. 2011;6(7):e22040. [Crossref]
21. Xiang Z, Hrabetova S, Moskowitz SI, Casaccia-Bonnefil P, Young SR, Nimmrich VC, Tiedge H, Einheber S, Karnup S, Bianchi R, Bergold PJ. Long-term maintenance of mature hippocampal slices in vitro. J Neurosci Methods. 2000;98(2):145-154. [Crossref]
22. Schommer J, Schrag M, Nackenoff A, Marwarha G, Ghribi O. Method for organotypic tissue culture in the aged animal. MethodsX. 2017;4:166-171. [Crossref]
23. Tan GA, Furber KL, Thangaraj MP, Sobchishin L, Doucette JR, Nazarali AJ. Organotypic Cultures from the Adult CNS: A Novel Model to

Study Demyelination and Remyelination Ex Vivo. Cell Mol Neurobiol. 2018;38(1):317-328. [Crossref]
24. Ravi VM, Joseph K, Wurm J, Behringer S, Garrelfs N, d'Errico P, Naseri Y, Franco P, Meyer-Luehmann M, Sankowski R, Shah MJ, Mader I, Delev D, Follo M, Beck J, Schnell O, Hofmann UG, Heiland DH. Human organotypic brain slice culture: a novel framework for environmental research in neuro-oncology. Life Sci Alliance. 2019;2(4). [Crossref]
25. Andersson M, Avaliani N, Svensson A, Wickham J, Pinborg LH, Jespersen B, Christiansen SH, Bengzon J, Woldbye DP, Kokaia M. Optogenetic control of human neurons in organotypic brain cultures. Sci Rep. 2016;6:24818. [Crossref]
26. Verwer RW, Baker RE, Boiten EF, Dubelaar EJ, van Ginkel CJ, Sluiter AA, Swaab DF. Post-mortem brain tissue cultures from elderly control subjects and patients with a neurodegenerative disease. Exp Gerontol. 2003;38(1-2):167-172. [Crossref]
27. De Simoni A, Yu LM. Preparation of organotypic hippocampal slice cultures: interface method. Nat Protoc. 2006;1(3):1439-1445. [Crossref]
28. Doussau F, Dupont JL, Neel D, Schneider A, Poulain B, Bossu JL. Organotypic cultures of cerebellar slices as a model to investigate demyelinating disorders. Expert Opin Drug Discov. 2017;12(10):1011-1022. [Crossref]
29. Plenz D, Kitai ST. Generation of high-frequency oscillations in local circuits of rat somatosensory cortex cultures. J Neurophysiol. 1996;76(6):4180-4184. [Crossref]
30. Sieg F, Obst K, Gorba T, Riederer B, Pape HC, Wahle P. Postnatal expression pattern of calcium-binding proteins in organotypic thalamic cultures and in the dorsal thalamus in vivo. Brain Res Dev Brain Res. 1998;110(1):83-95. [Crossref]
31. Ito Y, Banno R, Hagimoto S, Ozawa Y, Arima H, Oiso Y. TNFalpha increases hypothalamic PTP1B activity via the NFkappaB pathway in rat hypothalamic organotypic cultures. Regul Pept. 2012;174(1-3):58-64. [Crossref]
32. Ostergaard K, Finsen B, Zimmer J. 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. 1995;103(1):70-84. [Crossref]
33. Holmes C, Jones SA, Greenfield SA. The influence of target and non-target brain regions on the development of mid-brain dopaminergic neurons in organotypic slice culture. Brain Res Dev Brain Res. 1995;88(2):212-219. [Crossref]
34. Foidl BM, Ucar B, Schwarz A, Rebelo AL, Pandit A, Humpel C. Nerve growth factor released from collagen scaffolds protects axotomized cholinergic neurons of the basal nucleus of Meynert in organotypic brain slices. J Neurosci Methods. 2018;295:77-86. [Crossref]
35. Kearns SM, Scheffler B, Goetz AK, Lin DD, Baker HD, Roper SN, Mandel RJ, Steindler DA. A method for a more complete in vitro Parkinson's model: slice culture bioassay for modeling maintenance and repair of the nigrostriatal circuit. J Neurosci Methods. 2006;157(1):1-9. [Crossref]
36. Ullrich C, Daschil N, Humpel C. Organotypic vibrosections: novel whole sagittal brain cultures. J Neurosci Methods. 2011;201(1):131-141. [Crossref]
37. Hutter-Schmid B, Kniewallner KM, Humpel C. Organotypic brain slice cultures as a model to study angiogenesis of brain vessels. Front Cell Dev Biol. 2015;3:52. [Crossref]
38. Baratta J, Marienhagen JW, Ha D, Yu J, Robertson RT. Cholinergic innervation of cerebral cortex in organotypic slice cultures: sustained basal forebrain and transient striatal cholinergic projections. Neuroscience. 1996;72(4):1117-1132. [Crossref]
39. Riley C, Hutter-Paier B, Windisch M, Doppler E, Moessler H, Wronski R. A peptide preparation protects cells in organotypic brain slices against cell death after glutamate intoxication. J Neural Transm (Vienna). 2006;113(1):103-110. [Crossref]
40. Ding Y, Li L. Lipopolysaccharide preconditioning induces protection against lipopolysaccharide-induced neurotoxicity in organotypic midbrain slice culture. Neurosci Bull. 2008;24(4):209-218. [Crossref]
41. Foehring RC, Guan D, Toleman T, Cantrell AR. Whole cell recording from an organotypic slice preparation of neocortex. J Vis Exp. 2011(52). [Crossref]
42. Yoon JJ, Nicholson LF, Feng SX, Vis JC, Green CR. A novel method of organotypic brain slice culture using connexin-specific antisense oligodeoxynucleotides to improve neuronal survival. Brain Res. 2010;1353:194-203. [Crossref]
43. Gahwiler BH. Organotypic cultures of neural tissue. Trends Neurosci. 1988;11(11):484-489. [Crossref]
44. Li Q, Han X, Wang J. Organotypic Hippocampal Slices as Models for Stroke and Traumatic Brain Injury. Mol Neurobiol. 2016;53(6):4226-4237. [Crossref]