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Organotypic Hippocampal Slices as Models for Stroke and Traumatic Brain Injury

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Abstract Organotypic hippocampal slice cultures (OHSCs) have been used as a powerful ex vivo model for decades. They have been used successfully in studies of neuronal death, microglial activation, mossy fiber regeneration, neurogenesis, and drug screening. As a pre-animal experimental phase for physiologic and pathologic brain research, OHSCs offer outcomes that are relatively closer to those of whole-animal studies than outcomes obtained from cell culture in vitro. At the same time, mechanisms can be studied more precisely in OHSCs than they can be in vivo. Here, we summarize stroke and traumatic brain injury research that has been carried out in OHSCs and review classic experimental applications of OHSCs and its limitations.

Keywords Organotypic hippocampal slice cultures · Stoke · Traumatic brain injury

Introduction

Organotypic brain slice cultures are commonly used in brain disease research because they provide unique advantages over in vivo and in vitro platforms [1]. They largely preserve tissue structures, maintain neuronal activities and synapse circuitry, and replicate many aspects of the in vivo context. Additionally, the system is much simpler than an in vivo animal model and can be manipulated by overexpressing or knocking down genes. Thus, gene functions and pathways can be studied as easily as they can be in an in vitro system [1, 2].

Slice systems have been successfully established from spinal cord (acute slice cultures) and various brain regions, including hippocampus, striatum, cortex, olfactory epithelium, thalamus, and cerebellum [1, 3–9]. Slice cultures from hippocampus are the most commonly used to investigate the effects of drugs on neurons, microglia, and astrocytes and to assess neurogenesis. Organotypic hippocampal slice cultures (OHSCs) can be grown acutely or for long periods (chronic slice culture). Chronic slice culture can represent brain development, including the patterns of gene regulation, protein expression, and synaptic activity of age-matched hippocampus in vivo [1, 10–12]. Therefore, long-term hippocampal slice cultures are increasingly used as models for neurodegenerative disease, traumatic brain injury (TBI), and stroke, and as drug screening platforms to identify novel therapeutics. In Fig. 1, we summarize the disease models and pathophysiologic processes that can be studied with OHSCs and provide a schematic diagram showing a simple process for preparing chronic OHSCs.

OHSCs are usually prepared from rats or mice at postnatal days 3–9 (PND3–9) [1, 13]. Brain tissue during this time has a high degree of plasticity and is resistant to mechanical trauma during the slice preparation. Rodents that are younger than PND3 are not suitable for long-term slice culture because the slices lose their morphological characteristics [14]. Slices from adult animals often undergo neuronal degeneration, although some researchers claim to have prepared slices from adult rodents and maintained them in culture for several weeks [1, 15, 16]. After the brain is removed from anesthetized animal and separated into two equal hemispheres, the tissues are chilled in ice-cold dissection medium consisting of minimal essential medium (MEM), 24 mM HEPES, and 10 mM Tris–

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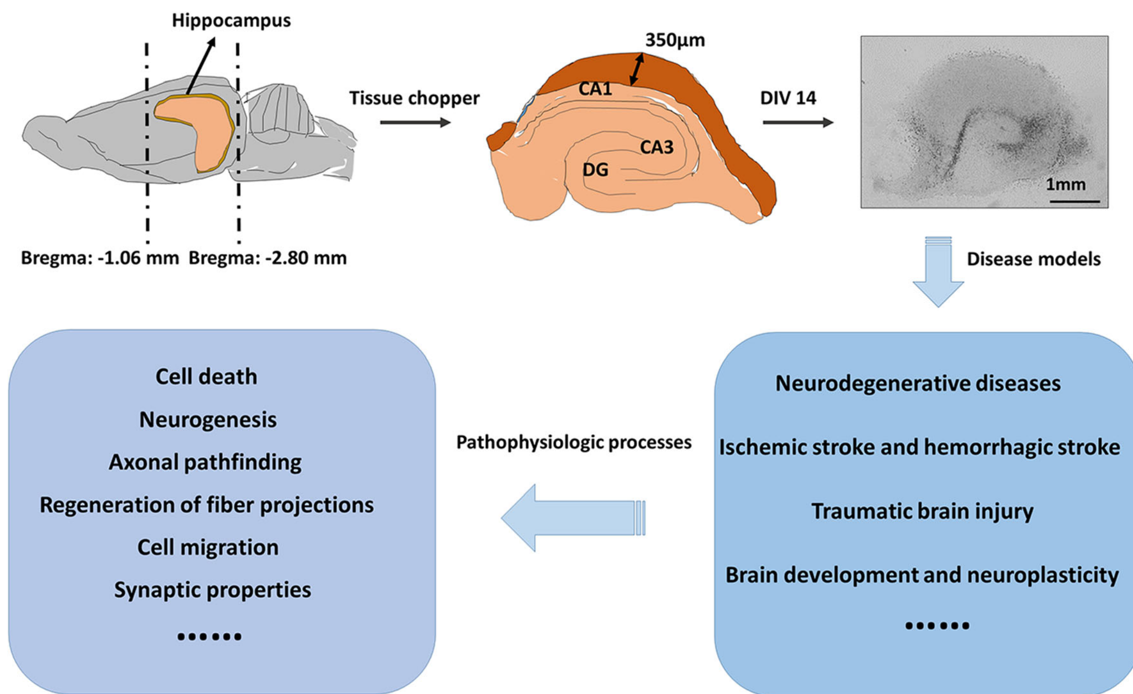

Fig. 1 The preparation and pathological models of chronic organotypic hippocampal slice cultures (OHSCs). Hippocampus is disected from mouse brains (from −1.06 to −2.80 mm relative to bregma) at postnatal days 3 to 9. OHSCs are prepared with a tissue chopper and are generally 350 μm in thickness. After being cultured for 10–14 days in vitro (DIV), the slices thin out and should have a healthy/integrated structure under brightfield microscopy. The disease models and pathophysiologic processes that can be studied with OHSCs are listed in the *boxes*. *DG* dentate gyrus

HCl or Hanks' balanced salt solution (HBSS), to help maintain viability and activity as much as possible [17]. Subsequently, a tissue chopper or a vibratome is used to cut the brain into 300–400 μm slices. The hippocampus is dissected carefully from slices for culturing. Two methods have been developed to maintain thin slices: the roller tube and the membrane interface method [13, 18–20]. The conventional roller tube technique is associated with preparation difficulties and large experimental viabilities [1]. Thus, membrane interface culture has become the most frequently used method [1]. In this method, brain slices are maintained on a porous membrane insert while the medium below the membrane provides nutrients to tissues by capillary action [1, 13]. The standard culture medium consists of 50 % MEM, 25 % heat-inactivated horse serum, and 25 % HBSS, supplemented with final concentrations of 25 mM glucose, 2 mM glutamine, or 12.5 mM HEPES and penicillin–streptomycin [17, 21–23]. The slices are incubated at 34–37 °C in a humidified atmosphere of 5 % CO_2 [23–25]. Mechanical lesions and alterations in metabolic state caused by release of enzymes and iron during tissue slicing are repaired during the first 6–18 days of culture [13]. After several weeks, the slices eventually thin down to 5–8 layers of cells (~100 μm) [13]. For use in experiments that mimic ischemia or excitotoxicity, a low-serum or serum-free medium may be used, or conditioned medium can be applied based on the experimental targets [26].

Healthy, undamaged slices are crucial for successful experiments and for minimizing experimental variation. On a healthy hippocampal slice, one should be able to easily identify cell bodies of pyramidal cells in CA1 and CA3 regions under a ×40 objective and smooth cell somata on the slice surface [13]. Various stains can also be used to help identify healthy slices. For example, propidium iodide (PI) is used to stain dead cells, and immunostains for markers of neurons and synapses can be used to identify lesions, certain healthy cell populations, and tissue architecture.

Experimental Applications of OHSC in Stroke and TBI

The technique of oxygen-glucose deprivation (OGD) mimics the conditions of ischemic stroke in vitro. A variety of neuroprotective mechanisms have been studied with the OGD model [25, 27, 28]. The hippocampal CA1 area is highly susceptible to OGD timing in OHSCs [29–31]. Variations in the time of preincubation, temperature, and cocultured cells strongly affect cell death [32–35].

PI is widely used to detect disrupted cells in slice culture. It binds to DNA in a nonspecific manner by penetrating the damaged phospholipid bilayers. Although PI cannot distinguish apoptotic and necrotic processes, it is accepted to

correlate well with the overall number of damaged cells [36, 37]. PI staining fails to distinguish the damaged cell population but provides an idea of the major damaged cell type by the unique structure of hippocampus. The concentration of PI usually used is 5 μg/ml, and 30 min to 1 h is an effective incubation period. Alternatively, damaged cells can be stained with Sytox Green (5 μM) for 30 min [38]. Cell death is calculated as 100×(Px–P0)/(Pmax–P0), where Px is the fluorescence intensity of slices at a certain time point, P0 is the background (baseline) fluorescence, and Pmax is the maximum fluorescence intensity of slices after a toxic dose of N-methyl-D-aspartate (NMDA), glutamate, or hypothermia, etc. [36, 39, 40].

Immunohistology is performed directly on the slices after different experimental treatments. Some groups prefer to perform the cryosection on OHSCs (20 μm) before immunodetection [41]. Ultrastructure, such as mitochondria, endoplasmic reticulum, and dendrites, can be observed by electron microscopy after traumatic insult [42].

Biochemical Characteristics

No special treatment is required to extract protein and mRNA from cultured slices except for pooling of samples. Five to six slices of rat hippocampus should be pooled [41, 43]. More slices are needed to extract proteins from mouse OHSCs.

An enzyme-linked immunosorbent assay (ELISA) kit is usually used to measure secreted proteins such as cytokines and chemokines in the culture medium of OHSCs. The overexpression of tumor necrosis factor α (TNFα), interleukin-6 (IL-6), and interleukin-1β (IL-1β) has been detected as early as 60 min after 60-min OGD [44].

mRNA microarrays can be performed and analyzed from slices subjected to hypoxia and OGD models [45]. Hypoxic preconditioning has been shown to upregulate apoptosis/survival-related genes, and isoflurane exposure has been shown to upregulate cell cycle/development genes such as *Egr* and *Pten* [45]. Proteins that regulate apoptosis, such as B cell lymphoma 2 (Bcl-2), P53, and murine double minute (MDM2), can be analyzed by Western blotting for evaluation of cell death pathways [45, 46].

Electrophysiologic Function

Organotypic slice culture permits long-term exposure to chemicals or drugs. Moreover, the chronic exposure may produce results that are opposite of those obtained in acute experiments [47]. After TBI is induced in hippocampal slice culture, the response amplitude, threshold intensity to obtain 50 % maximal response, and spontaneous oscillations are recorded [48, 49]. Stretchable microelectrode arrays (SMEAs) have been developed to record neuronal activity from multiple electrodes. Unlike typical electrodes, SMEAs deform with the tissue during TBI-induced stretching [49]. SMEAs can stimulate and detect electrical activity from cultured tissue without causing additional mechanical damage [50].

Neural Genesis (Axogenesis, Gliogenesis)

To determine the fate of newly generated neurons and glial cells, Strassburger et al. [41] detected proliferating cells by using 5-bromo-2-deoxyuridine (BrdU) and by labeling with specific cell markers sequentially. At 1 week after 40-min OGD, little endogenous neurogenesis had occurred, as detected by doublecortin, the early neuronal marker; on the contrary, the majority of BrdU-positive cells were microglia or GFAP-positive cells [51]. When the injured OHSCs were treated with anti-inflammatory agents, neurogenesis was induced in the posterior periventricular zone at 6 days after 40-min OGD [41]. Sierra et al. [52] also showed that microglia in the hippocampus can regulate neurogenesis through phagocytosis. Moreover, neural progenitor cells can be grafted to the slice cultures to study chemokines that regulate their migration and to investigate survival [53], differentiation, synaptogenesis, and function of the transplanted cells [35, 54–56]. In comparison with neurogenesis, endothelial cell remodeling and angiogenesis have not been studied in OHSCs [57, 58].

Hypothermia

Mild hypothermia after OGD produces regional and time-dependent neuroprotective effects [59]. Gregersen et al. [60] cooled the slices with mild (33–34 °C), moderate (<25 °C), and profound hypothermia (<20 °C) to investigate the limitations of hypothermia after 60-min OGD. They found that the protective effect of mild and moderate hypothermia was time-dependent and that profound hypothermia increased cell death.

Coculture and Transgenic Mice

To study the role of microglia in neuroprotection and neurodegeneration, microglia can be depleted from OHSCs with chemicals such as saporin. The depletion of microglia was shown to increase neurodegeneration at 1, 7, and 14 days after 30-min OGD [61]. Conversely, microglia can be added to the slice cultures. Microglia provided a neuroprotective effect when applied up to 4 h after 40-min OGD [62]. Protein-dependent function can be studied by using shRNA to knock down a gene in OHSCs or by culturing slices from knockout or transgenic animals [53, 63–65].

Applications to Stroke

In 2000 and 2012, stroke was the second leading cause of death in the world (http://www.who.int/mediacentre/

factsheets/fs310/en/). Even with timely treatment, it still may cause severe long-term sequelae, including unilateral paralysis, awareness and memory problems, speech problems, limb pain, and even depression owing to onset and delayed neuronal cell death and secondary neuroinflammation. The pathogenic mechanisms, neuroprotection, and repair mechanisms of stroke need to be investigated, and potentially effective drugs need to be tested in preclinical or translational research.

Ischemic Stroke

Ischemic stroke accounts for approximately 87 % of all strokes and is the most well-studied stroke type. Because OGD can mimic the conditions of ischemic stroke in hippocampal slice cultures, which represent a complex mixed cell population, it has been commonly used in ischemic stroke studies since 1996 [66, 67]. Pathogenic mechanisms of acute and delayed neuronal loss that have been studied in OHSCs include cellular energy depletion, accumulation of extracellular glutamate and other excitotoxins, calcium overload, mitochondrial dysfunction, and oxidative stress [66]. CA1 pyramidal cells are most vulnerable to OGD; the CA3 and dentate gyrus regions are less vulnerable [66, 68]. OGD has been shown to induce apoptosis [69, 70], necrosis [71], and a mixture of both [72]. The differences in cell death type might depend heavily on the duration of OGD (short OGD tends to cause apoptosis, whereas longer OGD causes necrosis) and the oxygen concentration [73]. Studies in which different kinds of cell death are targeted have been carried out for years. Pretreatment with caspase inhibitor Ac-YVAD-cmk (1 h before until 24 h after OGD) protected against CA1 cell death and also prevented synaptic dysfunction [68]. Another caspase inhibitor, z-VAD-fmk, also provided a neuroprotective effect when administered during and after 30-min OGD [74]. However, inhibitors of poly (ADP-ribose) polymerase have no neuroprotective effect and have even caused more cell death when tested in OHSCs [70, 73, 74]. Although many groups have tried, it is not easy to replicate the typical time pattern of neuronal cell death after global ischemia with OGD in vitro or ex vivo: acute cell death in the dentate hilus followed by delayed cell death in the CA1. For now, OGD is a common ex vivo model used to study the molecular and cellular mechanisms of ischemic stroke and the effectiveness of neuroprotective compounds in OHSCs.

Application of glutamate is believed to mimic excitotoxicity that occurs as a consequence of ischemic stroke. Excess glutamate, which is released into the extracellular space by damaged cells, can overexcite inotropic and G-coupled metabotropic glutamate receptors and thereby accelerate calcium ion entry into the cell. Calcium can also cause the release of more glutamate. Glutamate can bind to two types of receptors: inotropic receptors [including NMDA, kainate, and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)] and metabotropic glutamate receptors. Metabotropic glutamate receptors can be excited by L-2-amino-4-phosphonobutyric acid (L-AP4), 1-amino-1,3-dicarboxycyclopentane (ACPD), and L-quisqualic acid (L-QA) [66, 75]. Exposing OHSCs to NMDA and AMPA causes CA1 pyramidal cell death [76, 77]. Neurons located in CA3 are particularly responsive to administration of kainic acid (KA) and demoic acid [76–81]. Interestingly, different agonists induce different types of cell death. For example, KA caused necrosis but not apoptosis in OHSCs [80]. Antagonists of glutamate receptors have been tested in conjunction with OGD. Repeated studies have shown that treating OHSCs with MK-801, an NMDA antagonist, rescues most neurons from death during and after OGD [7, 82–86], but results regarding whether posttreatment with MK-801 protects against neuronal death have been conflicting [7, 86]. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) protected against damage from glutamate, and GYKI 52466 and 2,3-dihydro-6-nitro-7-sulfamoyl-benzo-quinoxaline (NBQX) showed protection against KA, AMPA, and ATPA [(RS)-2-amino-3-(3-hydroxy-5-tert-butylisoxazol-4-yl)propionic acid] toxicity [77, 87].

Calcium influx into the damaged neurons is a cause of cell death after stroke. Iron channels and activated NMDA, AMPA, and KA receptors are highly permeable to calcium [88–93]. Sodium influx through NMDA, AMPA, and KA receptors also causes calcium overload that is mediated by voltage-sensitive calcium channels [66]. Therefore, the efficacy of calcium channel blockers and tetrodotoxin, a sodium channel blocker, has been tested by PI staining and electrophysiology methods [67, 83, 94]. Importantly, both L-type and N-type calcium channel blockers have been shown to provide neuroprotection in vivo and in vitro, but it was not clear whether the protection was through the vasculature or neurons [67]. OHSCs provided a unique platform that enabled researchers to determine that the effect of an N-type calcium channel antagonist (omega conotoxin MVIIA) was mediated directly through neuronal calcium channels, whereas the effect of dihydropyridines (which block L-type calcium channels) might be mediated through vascular calcium channels or indirectly through actions in other brain regions [67].

Calcium influx leads to activation of the mitochondrial permeability transition pore (MPTP). The resulting depolarization of the mitochondria causes a loss of ATP production, an increase in reactive oxygen species (ROS), and damage to cytochromes in the electron transport chain [66, 95–98]. The increased presence of ROS and calcium within the mitochondria leads to lipid peroxidation and membrane damage [66]. Nitric oxide (NO) accumulates and reacts with superoxide anion to form peroxynitrite, which causes additional cell damage and leads to apoptotic and necrotic cell death [66, 97]. Mitochondrial inhibitors (3-nitropropionic acid), compounds that induce glutathione depletion (l-buthionine-sulfoximine),

and other reagents have been used to mimic the pathological process of ROS generation in OHSCs [66]. Neuroprotective compounds like RU486, an antagonist of progesterone and glucocorticoid receptors, have been shown to protect against the effects of ROS-induced cell death, including amyloid beta protein, hydrogen peroxide, and glutamate overloading [99]. This protective effect was independent of the presence or activation of glucocorticoid or progesterone receptors [99]. Genipin, the multipotent ingredient in gardenia jasmenoides fruit extract, was also able to reduce cell death stemming from ROS and reactive nitrogen species production [38]. In addition, mild hypothermia (31–33 °C) has been shown to protect against OGD-induced (60 min) neuronal death by reducing free radical production [100].

Damaged neurons undergo cellular degeneration after the onset of cerebral ischemic stroke. During this process, the injured neurons upregulate stress-related signaling pathways and secrete chemokines/cytokines that strongly activate nearby glial cells [101–103]. In response to the inflammatory signals, microglia/macrophages are recruited to the infarct area, where they contribute to phagocytosis of damaged neurons, formation of scar tissue, and secondary inflammation. The secondary inflammation potentially increases cerebral infarct size and worsens clinical outcome of patients with ischemic stroke [103–105]. It is commonly accepted that microglia/macrophages have two distinct activation phenotypes. The M1, or classically activated phenotype, is associated with secretion of inflammatory cytokines and generation of ROS, whereas the M2, or alternatively activated phenotype, is believed to secrete anti-inflammatory factors, phagocytize damaged neurons, and contribute to regeneration of injured tissues [106]. The ability to manipulate microglial/macrophage activation after stroke could affect ischemic stroke outcomes. D-JNKI1, a specific JNK inhibitor, was shown to decrease activation of microglia after 30-min OGD through the JNK pathway in neurons [103]. Additionally, the expression and activity of matrix metalloproteinase (MMP) 9 was increased after OGD (48 h) in microglia; treatment with either MMP inhibitor AG3340 (prinomastat) or minocycline reduced OGD-induced (48 h) gelatinolytic activity as well as neural injury [107]. Moreover, histone deacetylase inhibitors trichostatin A and suberoylanilide hydroxamic acid inhibited lipopolysaccharide-induced microglial activation by decreasing the secretion of IL-6, macrophage inflammatory protein-2, and NO. This effect may be mediated by the NF-κB pathway [108].

Hemorrhagic Stroke

Fewer studies have used OHSCs to model hemorrhagic stroke. Nicaraven is an agent that is especially beneficial in vasospasm or brain damage caused by subarachnoid hemorrhage (SAH) [109]. In the only study that used OHSCs to model SAH, nicaraven was reported to protect neurons from 30-min OGD and/or NMDA-induced cell death by inhibiting poly (ADP-ribose) synthase and scavenging free radicals [109]. To our knowledge, no published study has used OHSCs to investigate the pathophysiology of intracerebral hemorrhage. Hemoglobin, a main component of blood, has been reported to bind with NO and block long-term potentiation of CA1 neurons [110]. Other blood components that may have an impact on neuronal toxicity, such as thrombin, have been studied with OHSCs. However, such studies are usually considered to benefit ischemia, but not hemorrhage [111]. Thrombin has been reported to protect neurons from OGD at low concentrations (50 pM, 0.01 U/ml), but it reduced neuronal survival at higher concentrations (50 nM, 10 U/ml) [111]. The molecular mechanism by which thrombin causes injury may relate to FXa (perinuclear activated factor X), which catalyzes the conversion of prothrombin to thrombin in neural tissue after ischemia [112]. Protease nexin-1 and L-JNKI1 were able to prevent the neuronal injury caused by thrombin [113, 114].

Application to TBI

TBI is one of the most common neurologic disorders and is generally graded into mild, moderate, and severe. Based on a Centers for Disease Control and Prevention (CDC) report, an estimated 1.7 million people sustain acute TBI in the USA annually, and approximately 53,000 people die of TBI-related injury [115]. TBI consists of the primary injury, which occurs at the moment of impact, and secondary injury, which is characterized by brain swelling, hypoxia, hypotension, and a complex cascade of neuroinflammatory and metabolic events that lead to death or neurologic damage over time [116]. Although specific therapy for TBI is lacking, understanding the pathogenesis of TBI-induced brain injury is necessary. Here, we summarize studies that have used OHSCs to investigate TBI and the techniques and analytical tools that were used.

Induction of TBI in Slice Cultures

TBI can be mimicked in organotypic slice cultures by several methods. In one, a stainless steel cylinder (0.9 g) is rolled on the slices to mimic the primary traumatic injury of head-impact accidents [36]. In another model, a metal stylus or weight (0.137 g) is held 2 or 7 mm above the slice and then allowed to fall onto a localized area [36, 117]. This model induces secondary injury, enabling the researcher to follow the spread of cell injury, hypoxic damage, and brain swelling [118]. The third method is stretch injury, known to generate an equibiaxial strain injury [117, 119]. The frequency and speed of the strain are controlled by a linear actuator, linear encoder, and motion control board. The device allows up to 100 %

control of tissue strain and strain rate (up to 150 s^{-1}) [120]. Cell damage after injury closely correlates with strain [121]. Of note, although 5 and 10 % biaxial lagrangian strain induced minimal cell death, as determined by PI staining [122, 123], the maximal evoked response and the excitability of neural networks were decreased [48].

Cell Death (PI Staining)

PI uptake is used to indicate cell damage. When a mechanical TBI model was used, PI was increased significantly at 72 h. Application of dexmedetomidine by 2 h provided optimal protection, and MEK1-ERK was considered to be involved in mediating dexmedetomidine's protective effect [124].

Metabolism and Gene Expression

Most TBIs are mild [125]. Di Pietro et al. [117] compared the mRNA levels of various genes after a 10 % (mild) and 50 % (severe) stretch in slice cultures. They found that more genes were differentially expressed after mild TBI than after severe stretch, 210 vs 161 for upregulated genes and 789 vs 426 for downregulated genes. These genes were involved in a variety of cellular processes, such as multicellular organismal development, nucleosome organization, and chromatin assembly. Interestingly, severe stretch injury also activated neurodegenerative pathways such as the RhoA (Ras homolog gene family, member A) signaling pathway. The same investigators also investigated the metabolism of mild TBI [119]. HPLC results showed that ATP, ATP/ADP, and mitochondrial function were decreased, and gene microarray data indicated downregulation of transcriptional and translational genes at 24 h after mild TBI. These data indicate that a hibernation-type response was activated after mild TBI [119].

Activation of Microglia

After TBI, quiescent microglial cells with ramified processes become activated and take on an amoeboid morphology. In vivo, monocytes, neutrophils, and lymphocytes accumulate at the site of the lesion as a result of a breached blood–brain barrier [126–128]. These inflammatory cells release cytokines that contribute to secondary brain damage. Inflammatory cells also activate the complement cascade, which increases vascular permeability and secondary neuronal insults in both rodent and human brain [129, 130]. Activated microglia and blood-sourced macrophages are very hard to differentiate morphologically or by surface markers in vivo. Therefore, Bellander et al. [131] used OHSCs to evaluate the contribution of activated microglia to complement production after TBI. Their findings support the premise that microglia play a key role in complement activation after TBI, even in the absence of blood cells.

ROS

After TBI, secondary injury induces an extended cascade of pathological sequelae, including damage by ROS, reactive nitrogen species, and lipid peroxidation. Each contributes to damage of protein, DNA/RNA, cell membrane phospholipid architecture, and integrity of the blood–brain barrier [132, 133]. Various antioxidant agents have been shown to be protective in TBI models, including U-83836E, a potent and selective scavenger of LOO (*) radicals [134]; phenelzine, a scavenger of lipid peroxidation [135]; and deferoxamine, which inhibits iron toxicity [136]. Most of these investigations were performed in vivo. Recently, one ex vivo study on cultured rat hippocampal slices by Hughes et al. [38] showed that genipin protected against oxidative stress induced by *tert*-butyl hydroperoxide when it was administered at 1, 6, or 24 h after injury. No protection was detected when treatment was delayed by 36 h. ROS injury occurs soon after TBI. Huang and Huang [137] summarized 143 published in vivo and in vitro TBI studies and found that in most, ROS was sampled at 30 min to 1 h after acute TBI. However, ex vivo brain slice culture might provide a good model to investigate ROS as early as several minutes after TBI.

Epilepsy After TBI

TBI causes epilepsy and chronic seizures that are triggered by hyperexcitable networks [138, 139]. Extracellular electrophysiological recordings revealed that cortical oscillatory activity after TBI was suppressed [140]. Only 28 % of slices showed evoked activity 48 h after TBI, and the network activity recovered to baseline at 15 days after TBI [140]. The level of cyclooxygenase-2 and prostaglandin E2 (PGE2) increased after TBI [47]. In contrast to acute, postsynaptic application of PGE2, which decreases excitatory synaptic transmission in cultured slices, long-term (48 h) exposure to PGE2 upregulated presynaptic excitatory synaptic transmission, which may evoke a cascade of events that leads to epileptogenesis [47].

In early studies of TBI in OHSCs, researchers investigated fiber sprouting and functional regeneration. GAP-43 (growth-associated protein-43), a marker of axonal growth, was expressed by newly sprouted axons after transection of the CA3-CA1 transition [141]. Elevated local connection by increased presynaptic boutons caused hyperexcitability, which contributed to the genesis of seizures. TBI induced secretions of neurotrophic factors such as epidermal growth factor, brain-derived neurotrophic factor, and glial cell-derived neurotrophic factor [142, 143]. These factors might promote axonal connections by improving sprouting [144, 145]. Experiments in slice cultures have recently shown that interfering with the actions of these factors to reduce axonal sprouting might

reduce the risk of hyperexcitability and epilepsy development [146].

Conclusion, Limitations, and Prospects

Chronic hippocampal slice cultures have become a valuable platform to study both normal and diseased brain functions. Because OHSCs preserve the entire structure of hippocampus as it relates to neurogenesis, synaptogenesis, and axogenesis, it can be used as an ex vivo model of stroke and TBI to investigate not only acute neuronal cell death, but also neurogenesis and neuroplasticity [1]. As discussed in this review, OHSCs have a number of advantages as an ex vivo system. It is a fast, simple, and precisely controlled system; it preserves the three-dimensional neuronal network and therefore is closer to an animal model than is cell culture; it reduces the number of animals needed; slices can be cocultured with slices from different brain tissue or with specific cell populations to assess the interactions between them, and the effects of compounds and drugs can be investigated without concern about their ability to pass through the blood–brain barrier [1, 147].

Compared to the tri-culture system that artificially assembles various cell types (endothelial cells, pericytes, and astrocytes or endothelial cells, neurons, and astrocytes) in vitro, OHSCs contain all cell types and can be used to study cell-cell interactions under a variety of pathologic conditions. However, as an ex vivo model, the OHSC system shares many disadvantages with in vitro systems. For example, it lacks the influence of factors such as blood perfusion, cerebrovascular autoregulation, intracranial pressure, and neurovascular coupling, which are involved in pathophysiology of both stroke and TBI. Moreover, OHSC may have limitations for the study of calcium ions after stroke or TBI given the multiple homeostatic processes (Na^{+}-Ca^{2+} exchange, mitochondrial and endoplasmic reticular calcium accumulation, protein binding, etc.) involved in the maintenance of intracellular calcium, especially when compounded by mitochondrial abnormalities. Importantly, OHSCs are only suitable for examining the short-term effect of drugs on various injuries because the slices cannot survive for more than 3 weeks in vitro and will die within 3 days after intense or prolonged injury. It is also important to remember that the results obtained from OHSCs are only an approximation of what occurs in hippocampus of the mature animal brain. Studies have shown that neurons in OHSCs have more branches, higher order of dendrites, and more complicated synapses than those in acute slices [11]. Our group also observed that the morphology of astrocytes and microglia in OHSCs differ from that in frozen brain sections. Considering the cell reprogramming that could occur in an artificial environment with environmental pressures that differ from those in vivo, potential epigenetic changes in protein expression should make researchers evaluate their results more carefully. Although acute OHSCs may be more representative of mature brain than chronic OHSCs, acute slices have been used mostly for electrophysiology studies [148], rather than the examination of pathologic aspects described in this review. Only a few studies have reported using acute slice systems for stroke and TBI research [149–151]. Moreover, acute slices might have their own limitations. A recent study [152] indicated that astrocytes in acute slices exhibit structural and functional differences from those in vivo, including upregulated expression of GFAP, nestin, connexin 43, and AQP4; increased interstitial space volume; and different Ca^{2+} responses. Thus, acute and chronic OHSCs have their own advantages and disadvantages with regard to stimulating in vivo conditions. Of course, in vivo animal models are still ultimately necessary to evaluate the anatomic and functional outcomes of a therapeutic strategy.

Although most molecular biology techniques can be used in OHSCs with minor modifications, technical difficulties need to be resolved, such as how to quantify the morphology scientifically with 5–7 layers of cells, how to extract proteins with fewer slices, and how to decrease the variation in PI staining. Additionally, it is not yet clear whether gender and age of the pups, percentage of serum in the medium, and in vitro culture time affect certain experimental results.

To date, a limited number of studies have used OHSCs to study TBI, and even fewer publications focus on hemorrhagic stroke. Given its advantages, it might be beneficial to use this valuable platform more often for TBI and hemorrhagic stroke research.

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