BENCHMARK
Journal of Cellular Biochemistry 117:559–565 (2016)

Journal of Cellular Biochemistry

Comparative Analysis of Human and Rodent Brain Primary Neuronal Culture Spontaneous Activity Using Micro-Electrode Array Technology

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ABSTRACT

Electrical activity in embryonic brain tissue has typically been studied using Micro Electrode Array (MEA) technology to make dozens of simultaneous recordings from dissociated neuronal cultures, brain stem cell progenitors, or brain slices from fetal rodents. Although these rodent neuronal primary culture electrical properties are mostly investigated, it has not been yet established to what extent the electrical characteristics of rodent brain neuronal cultures can be generalized to those of humans. A direct comparison of spontaneous spiking activity between rodent and human primary neurons grown under the same in vitro conditions using MEA technology has never been carried out before and will be described in the present study. Human and rodent dissociated fetal brain neuronal cultures were established in-vitro by culturing on a glass grid of 60 planar microelectrodes neurons under identical conditions. Three different cultures of human neurons were produced from tissue sourced from a single aborted fetus (at 16–18 gestational weeks) and these were compared with seven different cultures of embryonic rat neurons (at 18 gestational days) originally isolated from a single rat. The results show that the human and rodent cultures behaved significantly differently. Whereas the rodent cultures demonstrated robust spontaneous activation and network activity after only 10 days, the human cultures required nearly 40 days to achieve a substantially weaker level of electrical function. These results suggest that rat neuron preparations may yield inferences that do not necessarily transfer to humans. J. Cell. Biochem. 117: 559–565, 2016.

KEY WORDS: MICRO ELECTRODE ARRAYS; PRIMARY NEURONS; BRAIN ELECTROPHYSIOLOGY; CULTURED PRIMARY NEURONS; NEURON ELECTRICAL ACTIVITY

Electrophysiology studies involving live neurons or brain tissue slice preparations are essential for investigating the electrical and functional development of the early mammalian brain. Not much is known about human fetal neuron development, the main limiting factor being the difficulty of making direct recordings of electrical activity of live human fetal neurons. Much of what is known is derived from two categories of electrophysiology experiments. The first, in vitro human studies, use tissue from postmortem fetuses in their 20th week, when gestation is about 50% complete. Electrophysiology of individual neurons can be studied via patch clamp (with limited options for studying network behavior), and histology can be studied using fixed tissue preparations. The second category, in vitro and in vivo animal studies, use in vitro rodent brain tissue or live dissociated cells, thus facilitating the study of neural networks. However, it is unclear to what extent findings in rodent preparations may be generalized to humans. Although, some similarities in the chemical, electrical and metabolic activity of rodent and human brain tissue have been demonstrated [Kolb, 1984], more sophisticated network electrophysiology has not been adequately studied. This work investigates that relationship by making Micro Electrode Array (MEA) preparations from dissociated primary neurons from both human and rat fetuses.

BACKGROUND

Electrical activity of early neurons is essential for brain development. Early spontaneous electrical activity is thought to guide the most fundamental processes of nervous system development, including neuronal migration, axonal outgrowth, transmitter

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Manuscript Received: 11 August 2015; Manuscript Accepted: 12 August 2015
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 18 August 2015
DOI 10.1002/jcb.25312 • © 2015 Wiley Periodicals, Inc.

phenotype selection, dendritic patterning, activation of transmitter receptors, programmed cell death, and the development of mature ion channel types [Moody and Bosma, 2005; Spitzer, 2006]. Just like adult cortical pyramidal neurons, human fetal neurons spend time in both quiet states and active states. In the past, the only available technique to investigate the ability of early neurons to generate spontaneous activity had been patch clamp recordings of post-mortem fetal tissue. However, the introduction of the Micro Electrode Array for making dozens of simultaneous recordings of rodent neuron cultures or brain slices has markedly advanced the field. These rodent networks have been used as a model for studying human brain properties and disorders. However, it has not been established to what extent findings in rodent cultures can be generalized to humans. This would require a direct comparison between rodent and human neurons grown under the same in vitro conditions, which, to our knowledge, has never been carried out before.

Early brain activity has long been an active area of study, with numerous reports showing the presence of complex brain activity in mammalian embryos before the nervous system is fully developed. This early activity is thought to be fundamental in regulating various processes that take place even before the onset of sensory-evoked activity [Fields, 1994; Khazipov and Luhmann, 2006; Moore et al., 2011]. For instance in [Arnold et al., 2005], the authors investigated long-term potentiation of hippocampal CA3–CA1 synapses and found that neurons cultured on MEA dishes display a simple transcription and protein synthesis-dependent form of plasticity modulated by electrical activations. In addition, it is well known that the formation, modification and repair of synaptic connections are guided by neuronal activity [Shatz, 1990; Fields and Nelson, 1992]. It is also well established, that genetic programs alone cannot provide sufficient information to specify the wiring and rewiring of individual synaptic connections with adequate precision within the nervous system. Electrical activity therefore strengthens, weakens, and breaks synaptic connections and plays a role in regulating growth cone motility, thus affecting neurite outgrowth and synaptogenesis [Fields, 1994].

RODENT BRAIN PREPARATIONS

Neural electrophysiology studies have traditionally been performed using rodent brain tissue for both in vivo and in vitro applications. Typically, dissociated neurons and brain slice preparations from rats are collected from 18-day-old rat embryos. Since the rat gestational period is 21 days, embryo development is approximately 86% complete at the time of dissection.

The electrical characteristics of rat dissociated cortical neurons have been extensively described in previous works [Chiappalone et al., 2006; Shahaf and Marom, 2001; Marom and Shahaf, 2002]. The main characteristics of such preparations are bursting and synchronized activity patterns occurring almost simultaneously over all electrodes in the network. These characteristic behavioral patterns typically begin after 8–12 days in vitro (div) and are present throughout the lifespan of these neuron networks. This bursting activity has been investigated extensively because of its resemblance to neural events typically associated with abnormal brain activity in humans such as epilepsy, meningitis or stroke. Network properties such as timescales of burst synchronization [Eytan and Marom, 2006] and burst propagation velocity [Maeda et al., [1995] have been investigated. The ability of electrical stimulation to control network plasticity [Wagenaar et al., 2005] and [Wagenaar et al., 2006], excitability [Bologna et al., 2010], and directionality [Novak and Wheeler, 1989] have also been studied extensively.

HUMAN BRAIN PREPARATIONS

Human brain tissue studies typically use post-mortem fetal brain preparations collected from fetuses in the 20th gestational week, when gestation is about 50% complete. Electrophysiology has been typically studied via patch clamp, even though this technique limits simultaneous neuronal recordings to approximately ten, thereby limiting the observability of network phenomena [Moore et al., 2011]. Patch clamp recordings are further limited by the inherent difficulty in regulating a number of relevant environmental conditions to which neural tissue is sensitive such as medium composition, serum deprival, increased partial oxygen tension, temperature, pH and, importantly, CO_2 levels. In contrast, because of the physical size of the recording apparatus, MEA recordings can be made inside a temperature and CO_2 controlled incubator, thus controlling environmental factors to a much higher degree of precision. Finally, in addition to electrophysiology, human fetal anatomy and histochemistry have also been studied (using fixed tissue slices) [Corlew et al., 2004], [Moody and Bosma, 2005], [Allène et al., 2008], [Yuste et al., 2007], and [Garaschuk et al., 2000] although functional electrical characterization of young human neurons is currently unavailable.

MATERIALS AND METHODS

CELL CULTURE PREPARATION

Primary human neuronal cultures were collected from brain tissue obtained from fetuses aborted at the beginning of the second trimester of gestation. Human primary neurons were provided by Temple University's Comprehensive NeuroAIDS Center. Fetal brain tissue (gestational age 16–18 weeks) was obtained from elective abortion procedures performed in full compliance with National Institutes of Health and Temple University ethical guidelines.

The tissue was washed with cold Hanks balanced salt solution (HBSS) and meninges and blood vessels were removed. For primary neuronal isolation, tissue in HBSS was digested with papain (Sigma–Aldrich, St. Louis, MO) for 30 min at 37°C. The tissue was further dissociated to obtain single-cell suspensions by repeated pipetting. Dissociated neurons were plated at a density of 5,000 cells/uL in neurobasal media with B27 supplement, horse serum, and gentamicin (Invitrogen, Waltham, MA), on MEA dishes previously coated with Polyethyleneimine solution and then laminin to promote cell adhesion. Cultures were kept in a cell culture incubator at 5% CO_2 at 37°C.

After approximately 1 h, neurons were re-fed with the same neurobasal media. Twenty-four hours later, cultures were re-fed with a complete change of neurobasal media without horse serum. Purity of cell type specific cultures was assessed by immunolabeling for cell-type specific markers. Although the number of astrocytes,

oligodendrocytes, and other non-neuronal cell types was minimized during plating, those cells were allowed to proliferate during culture.

During the experimental sessions, the cultures were transferred to a dedicated acquisition incubator in which the same environmental conditions were maintained. To reduce thermal stress to the cells, MEAs were kept at 37°C by means of a controlled thermostat. Half of the culture medium was changed twice per week, immediately after the recording sessions in order to maximize recovery time until the next recording session.

Three cultures were created using neural tissue from a single fetus. Culture 1 yielded 25 acquisitions starting on div 9, culture 2 yielded 24 acquisitions starting on div 17, and culture 3 yielded 26 acquisitions starting on div 12.

MEA RECORDINGS

Electrophysiology experiments were carried out utilizing micro-electrode arrays (60MEA 200/30iR-Ti-gr, Multi Channel Systems, Runtgen, Germany) consisting of 59 TiN/SiN planar round electrodes (30 um diameter; 200 um center-to-center inter-electrode intervals) arranged in a square grid without corners. A single larger electrode served as the ground node, replacing one recording electrode. All dish chambers were sealed with a gas permeable Teflon membrane to prevent contamination and evaporation. The activity of all cultures was recorded using the MEA60inv System (Multi Channel Systems, Reutlingen, Germany). Continuous voltage signals were recorded from each electrode, amplified 1,200x, sampled at 20 kHz with 14-bit (74.5uV/bit). Then the acquisition files were saved through the data acquisition card and MC_Rack software (Multi Channel Systems) installed on the acquisition computer. Data analysis was performed off-line using MC_Rack software, (Multi Channel Systems) and MATLAB (The Mathworks, Natick, MA).

Every experimental session was composed of a 30-min spontaneous activity recording. Every recording started 10 min after placing the dish onto the amplifier to give the neurons sufficient time to recover from mechanical stress due to movement and to get acclimated to the new conditions. Experimental sessions were typically conducted twice per week. Once spontaneous activations were observed regularly, (approximately 10 div for rat cultures, 50 div for human cultures), a brief stimulation protocol, comprising 120 biphasic pulse pairs per electrode over the course of 1 h, was delivered after each recording session.

OFF-LINE SIGNAL PROCESSING

The raw continuous voltage traces were band-pass filtered to enhance the spiking components of the signals. The band-pass filter comprised a high-pass 2nd order Butterworth filter at 180 Hz and a low-pass 2nd order Butterworth at 3 kHz. The spike detection threshold was individually set for each channel at −6 times each band-pass filtered signal′s standard deviation (as measured by MC_Rack software) within a 500 ms window. To reduce the possibility of detecting duplicated spikes a detection refractory period of 1 ms was implemented. Furthermore, during the spike detection, an operator visually inspected the data to make sure that no noisy channels or artifacts were mistakenly detected as neural spikes.

The detected neural spikes were used to measure network electrical activity and to quantify the time course of evolution of synaptic connections. The principal metric used was the array-wide spike detection rate (ASDR), defined as the total number of spikes detected per unit time, summed over all electrodes in the array. In order to concisely display and quantify the experimental results with respect to each culture over the course of multiple experimental sessions, we chose to average the ASDR over each experimental session to derive a single metric that was representative of the overall network activity.

RESULTS

Figure 1 summarizes the three cultures' electrical activity versus time. The top panel displays the average spike count measured as the number of spikes per time unit. The bottom panel represents the average active electrode number over the whole network. It can be seen that regular neural activity for the three human cultures arises after 39, 34, and 37 div, respectively. This active stage of the cultures' functional activity follows a preliminary developmental period in which these cultures do not display any significant electrical activity patterns. In contrast, the control rodent preparations display mature and stable functional activity after only 10–12 div, as shown in Figure 2.

In addition to the latency of the onset of culture activity, differences were also observed in the nature of the network behavior. Whereas rat neuronal cultures have been widely characterized by their synchronized bursting behavior [Maeda et al., 1995; Beggs and Plenz, 2003; Corlew et al., 2004; van Pelt et al., 2004; Chiappalone et al., 2006; Rolston et al., 2007; Napoli et al., 2014] this phenomenon was not observed in any of the three human cultures, even after the onset of electrical activity. For example, Figure 3 displays representative synchronous network-wide activations from dissociated rat neurons at 96 div. Synchronized activations are indicated by the vertically aligned pixels. In contrast, Figure 4 displays a typical MEA recording from dissociated human neurons at 47 div. Not only are activations sparse across the MEA dish, synchronous activity and bursting are also largely absent.

The human MEA cultures were examined under the microscope for evidence of failed plating or poor culture development. Figure 5 shows one of the human dishes at 10 div. The figure clearly shows robust evidence of neuronal culture health including axon and soma growth. However, despite a clearly successful plating process, the formed synapses do not exhibit any functional activity for at least 30 more days on average.

DISCUSSION

The MEA recordings of dissociated in vitro human primary neurons presented here differ from those of rats in two key ways. First, whereas cultured rat neurons show organized and spontaneous electrical activity after only 10–12 days in vitro, human neurons take nearly 40 days to begin firing. Secondly, the human neurons in this study fired only sporadically with a complete absence of coordinated network activity. In contrast, the control rat neurons demonstrated robust activations characterized by coordinated synchrony across the network. These results are relevant because they suggest that

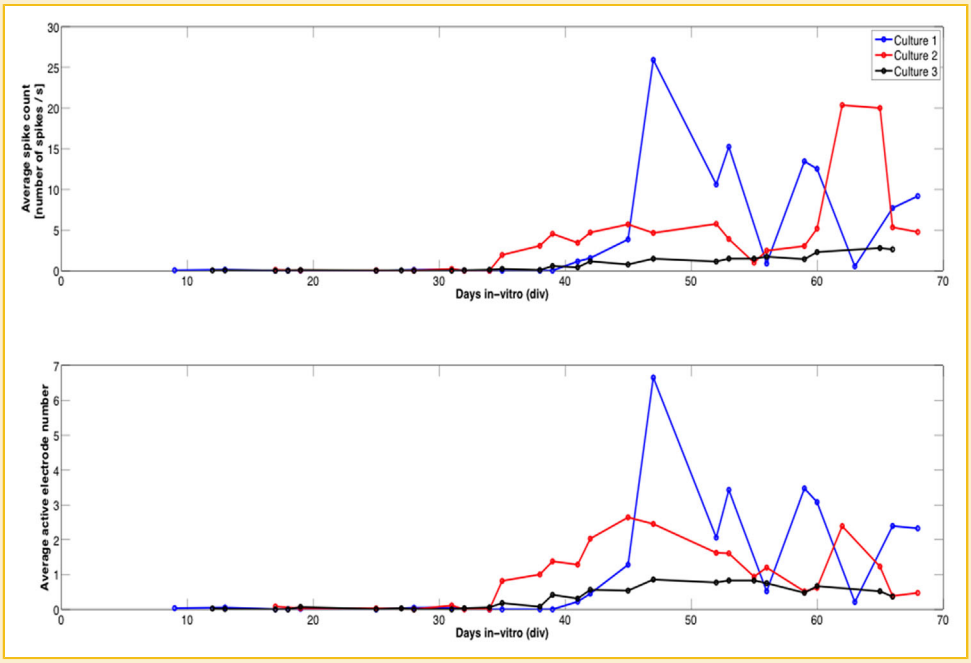

Fig. 1. Dissociated human neuron temporal evolution. (Top) Mean firing activity. (Bottom) Mean number of active electrodes.

findings from research studies performed on cultured rat neurons may not necessarily generalize to humans. Interestingly, studies on primary mouse neurons (from gestationally mature embryos) demonstrate culture activity similar to that observed in the rat cultures in the present study [Geissler and Faissner, 2012].

Although MEA cultures of primary human neurons have not been reported in the literature, there have been studies involving human neurons derived from stem cells [Pizzi et al., 2007, 2009]. In those studies, stem cells from a spontaneously aborted 10-week human fetus were differentiated and matured in vitro for 18 days before being cultured in

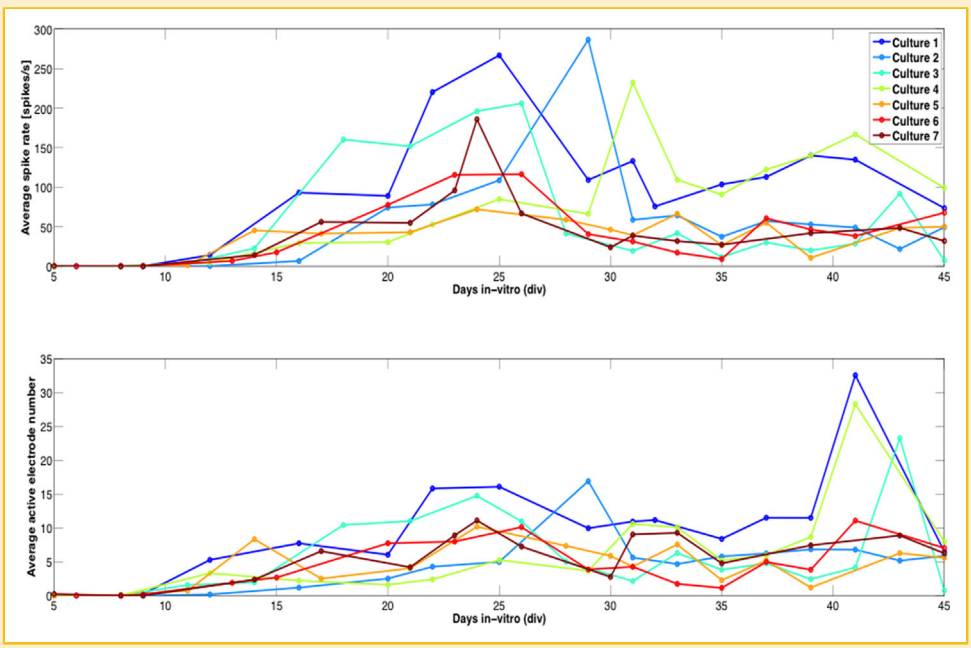

Fig. 2. Dissociated rat neuron temporal evolution. (Top) Mean firing activity. (Bottom) Mean number of active electrodes.

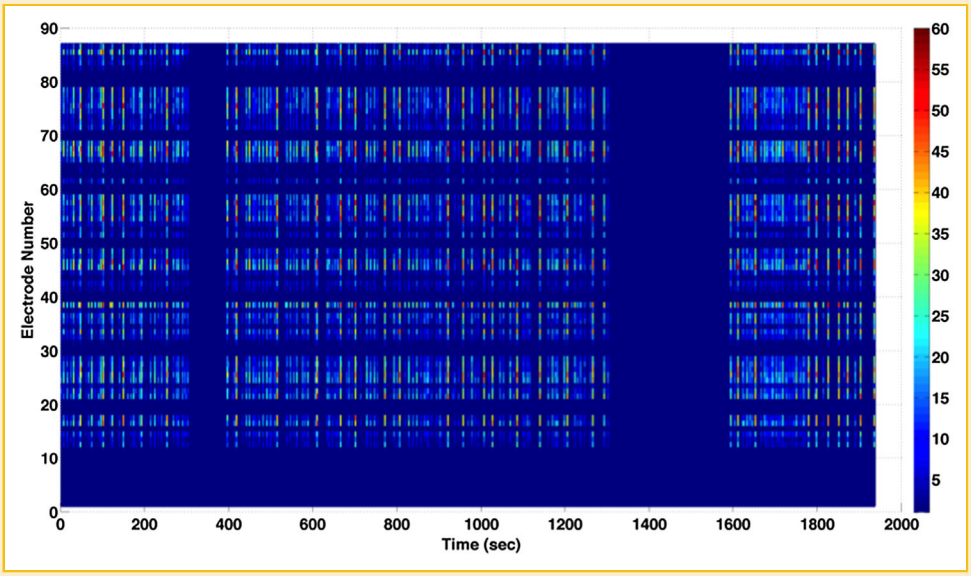

Fig. 3. Network activity from sample rat neuron culture at 45 div. The vertical lines indicate bursts and synchronous activity phenomena.

MEA dishes. The authors successfully used these preparations to create functional human neural networks that could differentially respond to various electrical stimuli. In another study, cultures of stem-cell derived human neurons were found to create spontaneously networks after 4 weeks in culture, with single spike activity recorded as early as the first week [Heikkilä et al., 2009]. However, in general, the properties of stem cell-derived human neuron cultures have been shown to be sensitive to the details of the particular induction protocol [Stice et al., 2006; Milet and Monsoro-Burq, 2012]. In this context, it is unclear to what extent findings based on stem-cell derived human neurons can be compared to those from primary neurons.

A number of hypotheses potentially explain the observations made in this study. One possibility is that the human neurons used in this study, being relatively less gestationally mature than their rodent counterparts, have not yet started to express genes that code for specific membrane proteins involved in the action potential

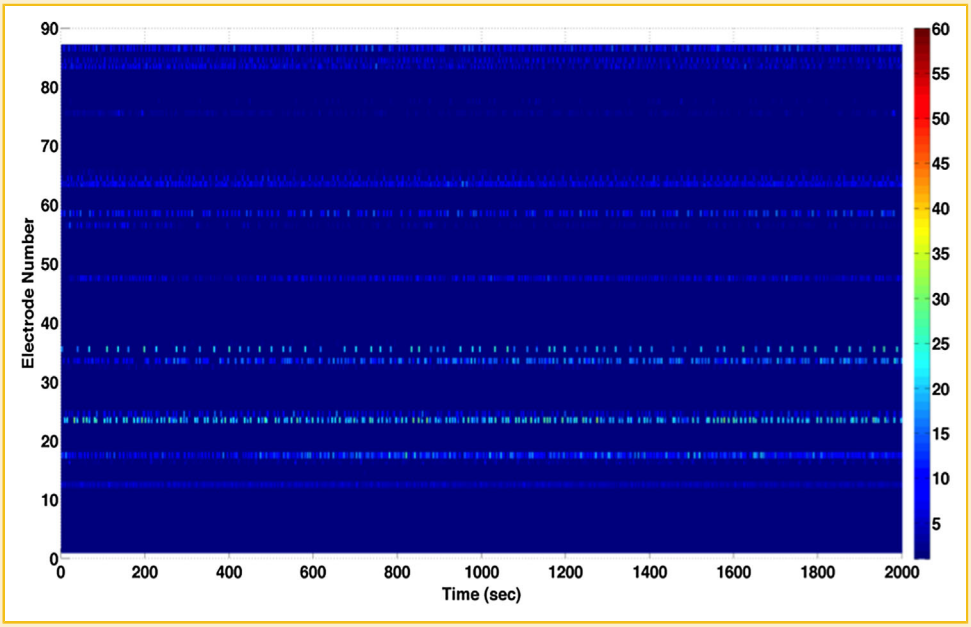

Fig. 4. Network activity from sample human neuron culture at 47 div. Bursts and synchronous activity are not characteristic of these recordings.

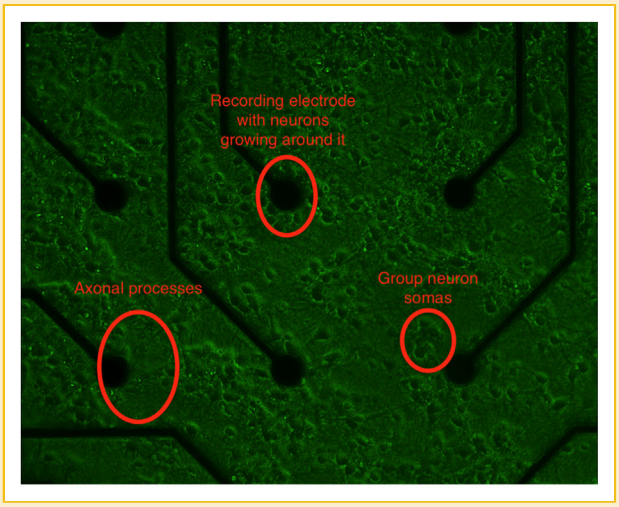

Fig. 5. Microscope image of dissociated human neurons 5 days after plating.

process. If true, this hypothesis may suggest new limitations on the timecourse of human cortical functional development. Another possibility is that the human cultures are more sensitive to the role of glia and other ancillary cell phenotypes than are rodent cultures. In both sets of cultures, the proliferation of non-neural cells was not externally regulated and instead occurred at a self-determined rate. A third hypothesis is that the extracellular environment in the human preparations takes longer to become sufficiently rich to support regular neural firing.

Interestingly, it was observed that changing the culture medium had significant effects on the spontaneous neuronal activity recorded from networks of dissociated human neurons. Specifically, the recordings show that after complete medium change, the regular network activity was suspended for up to 5 days before returning to previous activity levels. Subsequently, the effects of a half medium change were also tested and the networks' recovery time was reduced to 2–3 days. In contrast, medium changes in the rodent cultures produced increases in firing activity that typically lasted from 4 to 10 h.

CONCLUSION

This work presents a direct comparison of network electrophysiology in MEA cultures of human and rat neurons. Whereas the rat neuronal cultures formed robust functional electrical networks over the course a week, the human cultures took as many as 7 weeks to produce weak, asynchronous network activity. This work suggests that findings from research studies on rat neuronal cultures may not generalize to humans.

ACKNOWLEDGEMENTS

Human Primary Neurons were provided by Temple University's Comprehensive NeuroAIDS Center (P30 MH09217). Experiments and cell culturing were performed in the Department of Neuroscience Center for Neurovirology Comprehensive NeuroAIDS Center, School of Medicine, Temple University under the auspices of Dr. Kamel Khalili.

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