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ORIGINAL RESEARCH PAPER

Prolonging life in chick forebrain-neuron culture and acquiring spontaneous spiking activity on a microelectrode array

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Abstract Various types of animal neurons were cultured on a microelectrode array (MEA) platform to form biosensors to detect potential environmental neurotoxins. For a large-scale screening tool, rodent MEA-based cortical-neuron biosensors would be very costly but chick forebrain neurons (FBNs) are abundant, cost-effective, and easy to dissect. However, chick FBNs have a lifespan of ~14 days in vitro and their spontaneous spike activity (SSA) has been difficult to develop and detect. We used a high-density neuron-glia co-culture on an MEA to prolong chick FBN lifetime to 3 months with lifetime-long SSA. A remarkable embryonic age-dependency in the culture's morphology, lifespan, and most features of SSA signal was discovered. Our results show the feasibility of developing a chick FBN-MEA biosensor and also establish a new electrophysiological platform for functional study of an in vitro neuronal network.

Keywords Biosensor · Chick forebrain neuron · Lifespan · Microelectrode array · Spontaneous spiking activity

Abbreviations

SSA	Spontaneous spiking activity
MEA	Microelectrode array
FBNs	Forebrain neurons
SCNs	Spinal cord neurons
M199	Medium 199
DIV	Days in vitro
En	Embryonic day n
ACC	Active channel count, i.e., the number of active channels that are firing SSA signals from an MEA

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Introduction

Of the thousands of chemicals widely used in manufacturing, medicine, and commerce, more than 40 % have not been assessed for potential toxicity at a basic level, and less than 10 % have a full set of test data (Goldman and Koduru 2000; Wigle et al. 2007). Furthermore, thousands of new chemicals are annually

reviewed by the US Environmental Protection Agency. There is a pressing need for neurotoxic chemical screening on a large scale.

Microelectrode arrays (MEAs) are considered "a physiologically-based neurotoxicity testing platform for the 21st century" (Johnstone et al. 2010). Using MEA technology, various types of animal neurons from different regions of the central and peripheral nervous systems have been used to develop biosensors. These neurons include rodent cortex (frontal, auditory, and visual cortex), hippocampus, spinal cord, and dorsal root ganglion (Johnstone et al. 2010); human embryonic stem cell-derived neuronal cells (Ylä-Outinen et al. 2010); the NT-2 cell line derived from human pluripotent carcinoma stem cells (Laurenza et al. 2013); and chick spinal cord (Chiappalone et al. 2003). However, chick forebrain neurons (FBNs), an abundant and economical cortical-neuron resource, and thus a potential biosensor candidate, have rarely been investigated on an MEA platform, possibly due to brevity of their in vitro lifespan, which has generally been no more than 14 days (Heidemann et al. 2003; Tokioka et al. 1993), and the difficulty of detecting their spontaneous spike activity (SSA) (Pirlo 2009).

In a historical context, Louis et al. (1981) demonstrated rapid synaptogenesis from 4 days in vitro (DIV) to 7 DIV for E8 chick FBNs, but their eight-day lifespan in culture did not allow sufficient neuronal development. Tokioka et al. (1993), using a patch clamp, recorded both excitatory post-synaptic potentials (EPSPs) and inhibitory post-synaptic potentials (IPSPs) from E6, E8, E10, and E12 chick FBN cultures no more than 14 days old. This indicated that the synapses that had developed in vitro were functional. In 2003, Heidemann et al. (2003) made further efforts to culture chick E8 FBNs and compared eight types of culture medium. Medium 199 (M199) showed the best culture lifespan and fraction of cells developing axons to a more differentiated stage. However, the lifespans of their cultures were no more than 14 days. Pirlo (2009) was the first to culture chick FBNs on MEA, but could not detect SSA signals. It was unclear whether SSA did not emerge in his cultures or were undetectable for other reasons.

These previous investigators cultured chick FBNs at low densities ranging from 80 to 800 cells/mm^2. Because neurons thrive in a dense community, their brief lifespan may be largely attributable to this low density. Another cause of the brevity of the lifespan could be that most glial cells are generated after E10 in vivo (Tsai et al. 1981), so FBN dissection at E8 generally yields a relatively pure neuronal population that lacks glial support. Glial cells support healthy growth and maintenance of a neuronal network (Kandel et al. 2000), which is difficult to maintain long-term in vitro when glia are absent or reduced in number. We propose to prolong the lifespan of a chick FBN culture on an MEA to obtain a long-lasting SSA signal to address restrictions on use of large numbers of animals (e.g., rodents), which limit current neuron-based screening for environmental toxicants.

Materials and methods

Chick FBN culture on an MEA

Embryonic White Leghorn chick forebrains were dissected according to Heidemann et al. (2003). [The procedure of use of chick embryos were approved by the Clemson University Institutional Animal Care and Use Committee under Policy #23: Policy for the use of avian embryos, which conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, 8th Edition, 2011).] Forebrains were dissociated by a few periods of gentle titration and then trypsinized (0.25 % trypsin/EDTA) for 5–7 min at 37 °C. Dissociated FBNs were washed twice using a serum-containing medium and centrifuged at $\sim 1{,}000 \times g$ for 5 min. Cell pellets were resuspended in culture medium (described below) and plated on the surface of sterilized standard 60-electrode MEA chips (MCS-MEA-S1-GR, 200/30ir-Ti with internal ground, MCS GmbH, Reutlingen, Germany; see Fig. 1). Before plating, the MEAs were coated with 0.05 % polyethylenimine (PEI) overnight at 37 °C. After plating, each MEA was covered with a Teflon lid, kept in a 100 mm Petri dish, and placed in an incubator at 37 °C with 5 % CO_2 and 95 % humidity. The Teflon lid, permeable to gases but not water and bacteria, was used to prevent water evaporation and contamination (Potter and DeMarse 2001) because SSA is very sensitive to osmotic changes and is suppressed by hyperosmotic insult. To compare features of SSA between FBNs and spinal cord neurons (SCNs), the SCNs were dissected according to Kuhn (2003),

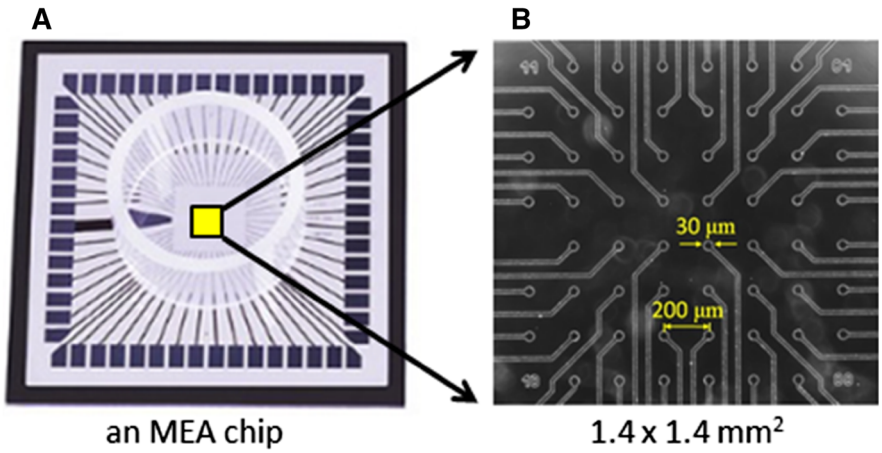

Fig. 1 **a** An MEA chip with 60 electrodes and **b** its center area (1.4 × 1.4 mm^2). Each electrode has a diameter of 30 μm, and the electrodes are 200 μm apart

dissociated, and cultured in the same way as the chick FBNs.

Strategies to prolong the life of chick FBNs in culture

(1) Cell plating at 1,000, 1,500, 2,000, 2,500, and 3,000 cells/mm^2 were tested. 3,000 cells/mm^2 was selected because it showed the most-even cell distribution. (2) Selected Medium 199 (M199, M4530, Sigma) was supplemented with 2 % B27 (17504-044, Gibco), 10 % (v/v) fetal bovine serum (FBS; Heidemann et al. 2003; Pirlo 2009), and 1 % antibiotic/antimycotic (Gibco). (3) Natural co-growth of glial cells was allowed. (4) To explore at which embryonic age chick FBNs can live the longest in culture and offer the longest-lasting robust SSA signals, chick forebrains from a wide range of embryonic days were used and compared. Tsai et al. (1981) reported that during in vivo embryonic development of the chick telencephalon (the later cortical structure of the cerebral hemispheres that derive from forebrains), the window for neuron generation is E4–E9, but most glial cells are generated after E10. To avoid potential problems, including that early embryonic age FBNs may not survive long due to lack of glial support and that the neurites of older FBNs can be more branched and more differentiated and thus more likely to be injured during dissection, we selected neurons from a few days of either side of E9 (E6, E8, E10, E12, and E15) to conduct the comparison. (5) For all cultures, medium was completely changed 24 h after cell plating; then 1/3 of the medium was changed once weekly for E6–E10 cultures and every three days for E12 and E15 cultures.

SSA signal monitoring and recording using an MEA

Because chick FBNs form a morphological neuronal network after approx. 3–4 days in vitro (DIV), SSA monitoring and recording were started at 3 DIV. From 3–6 DIV, MEA signals were recorded overnight; then, 10 min recording was conducted daily for the first three weeks. Later, recording was conducted every 3 days or weekly throughout a culture's lifespan. All recordings were performed while the MEA was held at 37 °C with 5 % (v/v) CO_2 and 65 % ambient relative humidity (Potter and DeMarse 2001). SSA signals from the MEA were amplified using an MCS 1060-INV amplifier (MCS GmbH, Reutlingen, Germany) and collected using MC Rack software (Version 4.3.0, MCS GmbH, Reutlingen, Germany) at a 25 kHz sampling rate.

Definition of SSA-related terms

SSA may take the form of sporadic spikes or organized spikes. A "spike" is an extracellular measurement of an action potential. If a channel shows five or more spikes per minute, it is an "active channel." A "burst" is a train of spikes organized into a high-frequency cluster. In our experience, bursts on individual channels always appear simultaneously across a large fraction of active channels. This network-wide bursting behavior—a synchrony of individual channel

bursting event(s)—is called "population burst(s)" (Pasquale et al. 2008). In this paper, if "population" is not specified before "burst," a "burst" refers to organized spike trains occurring at the individual-channel level.

Spike detection and burst detection

The threshold for spike (a downward pulse) detection was set at six standard deviations (SD) lower than the mean noise amplitude for each active channel. Three criteria determined a burst: (1) the minimum number of spikes within a burst was equal to or greater than 3; (2) the maximum interspike interval (ISI_{max}) within a burst was equal to or less than 100 ms; and (3) the minimum burst duration was equal to or greater than 10 ms. Spike detection was performed using MC_Rack and burst detection was achieved using a self-developed MatLab program (NeuroMEA).

Data analysis using NeuroMEA

NeuroMEA reads files recorded by MC_Rack, outputs endpoint data, and creates raster plots. The endpoint data include two groups: spike-related parameters and burst-related parameters. The former include mean spike rate, mean spike amplitude, and the maximum and minimum spike amplitudes. The latter include mean burst rate, percentage of spikes in burst, burst duration, interburst interval, spike frequency in burst, and interspike interval in burst.

Results

The in vivo sequence of neuronal and glial development was retained in vitro: we observed an obvious embryonic age-dependent culture morphology and lifespan. SSA signals were largely embryonic age-dependent.

Culture morphology

The culture's morphological features were described in terms of the following: (1) Whether cells aggregated and, if so, to what degree? (2) When and how quickly could glial proliferation be visualized? and (3) Was a culture able to be confluent and if so, how quickly? Table 1 (columns 2–4) summarizes these three aspects for E6–E15 cultures, respectively. Figure 2 shows phase-contrast images of representative E8, E10, and E12 cultures at 3 culture stages. Both Table 1 and Fig. 2 demonstrate the culture feature of distinct embryonic-age dependence. 10 % (v/v) FBS in M199 caused cell aggregation in E6–E10 cultures while 5 % (v/v) FBS reduced cell aggregation and improved the integrity of the cell monolayer; however, the SSA signals were compromised and lasted a shorter time (not shown).

As shown in Fig. 2, E8 cells aggregated extensively to form cell clusters intermingled with open spaces. Glial-cell proliferation was delayed in the E8 culture; these open areas filled slowly or did not become completely confluent. E10 cells formed cell islands that looked thinner (less severely aggregated) than the cell clusters in E8 culture. Glial-cell proliferation in E10 was faster than in E8, so the open areas filled gradually. E12 cells did not aggregate at all; when compared to the other cell groups, E12 cells formed a confluent cell monolayer easily and earliest, with full coverage of all microelectrodes.

Culture lifespan

Our data demonstrate (Table 1, column 5) that FBNs harvested at different embryonic ages had different lifespans in culture. Lifespan was determined by the DIV when the culture automatically detached from the MEA surface while it was still spontaneously active or when we ended the culture at an active channel count (ACC) of 7 or below. In the current culture setting in M199 with 10 % FBS, E8 and E10 cultures were able to achieve a lifespan of one month. Among all selected embryonic ages, E12 samples achieved the longest lifespan of 3 months.

Culture SSA

Onset of SSA signals

Timing of the onset of spikes, bursts, and population bursts varied according to culture density, FBS concentration, and medium types. The timing of the onset of these signals in our dense culture (plated at 3,000 cells/mm^2 in M199 with 10 % FBS) is summarized in Table 2; the *n* and *N* are the same as in Table 1. It should be noted that although a culture's morphology was embryonic-age dependent, the onset of SSA signals was

Table 1 Embryonic age-dependent morphological features and lifetime of chick FBNs in dense culture at plating density of 3,000 cells/mm^2 with 10 % FBS

Embryonic age (day)	Cell aggregation	Glial cell proliferation	Cell monolayer integrity	Longest lifetime (DIV)	*n* (Number of cultures)	*N* (Number of dissections)
E6	Severe	Delayed, slower	Hard to achieve confluence	21	24	8
E8	Severe	Delayed, slow	1/3 to 1/2 of cultures were confluent	29	20	6
E10	Moderate	Delayed, fast	Slow confluence	31	6	3
E12	No	No delay	Fast confluence	90	6	3
E15	No	No delay	Less confluent due to regional degeneration	Not determined (ACC $\leq$ 7)	6	3

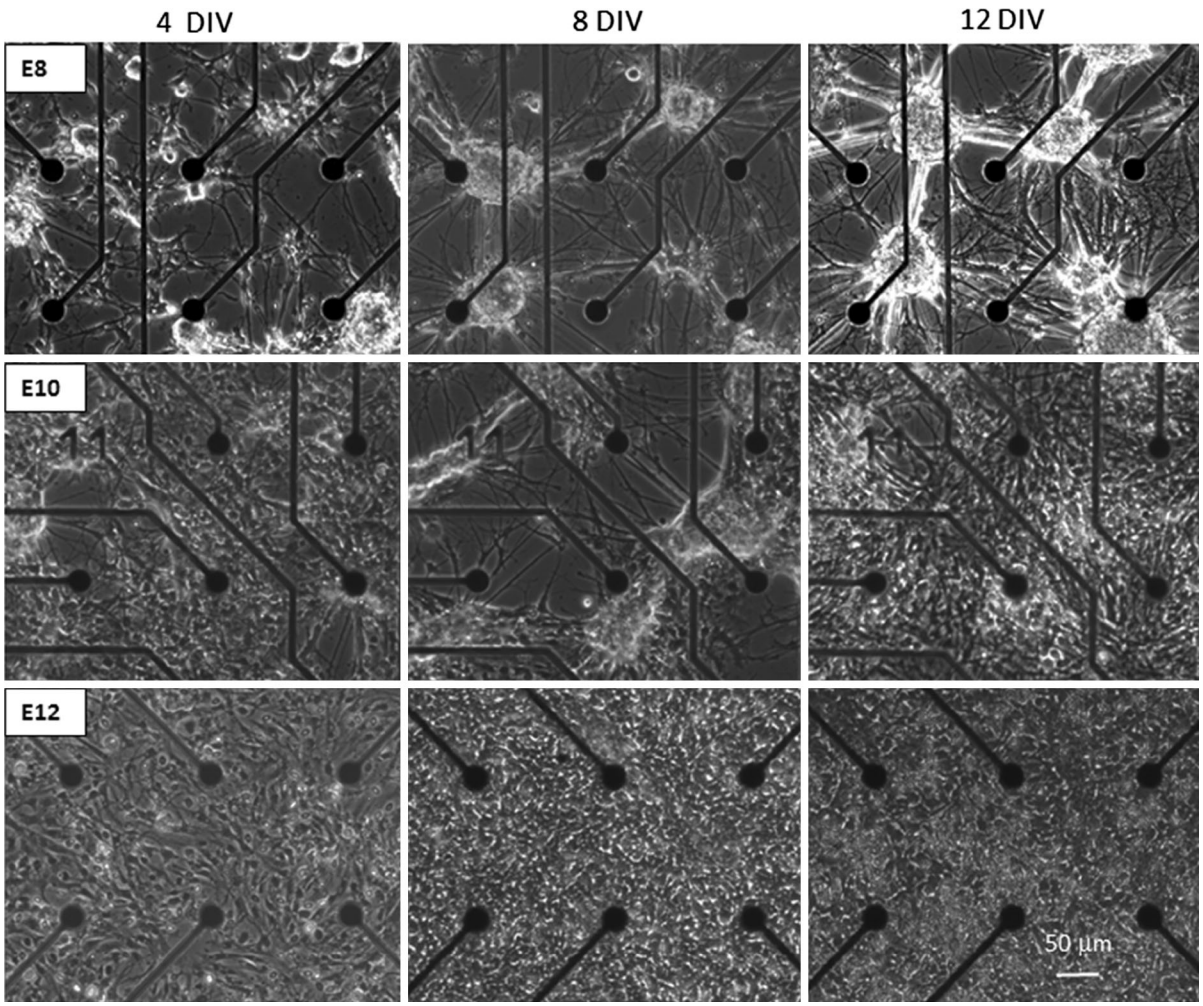

Fig. 2 Phase-contrast images (10×) of embryonic age-dependent morphological features of chick FBNs in dense culture at a plating density of 3,000 cells/mm^2 in M199 with 10 % FBS

Table 2 Occurrence of SSA-signal onset by DIV in dense cultures at a plating density of 3,000 cells/mm^2 in M199 with 10 % FBS

Embryonic age (day)	Spike		Bursts		Population bursts	
	Earliest detection (DIV)	Typical emergence (DIV)	Earliest detection (DIV)	Typical emergence (DIV)	Earliest detection (DIV)	Regular emergence (DIV)
E6	4	6–11	5	Varied greatly	5	Varied greatly
E8	4	5–7	5	5–8	5	5–8
E10	4	5–7	5	5–8	5	5–8
E12	4	5–7	5	5–8	5	5–8
E15	4	5–7	Occurred rarely		Did not develop	

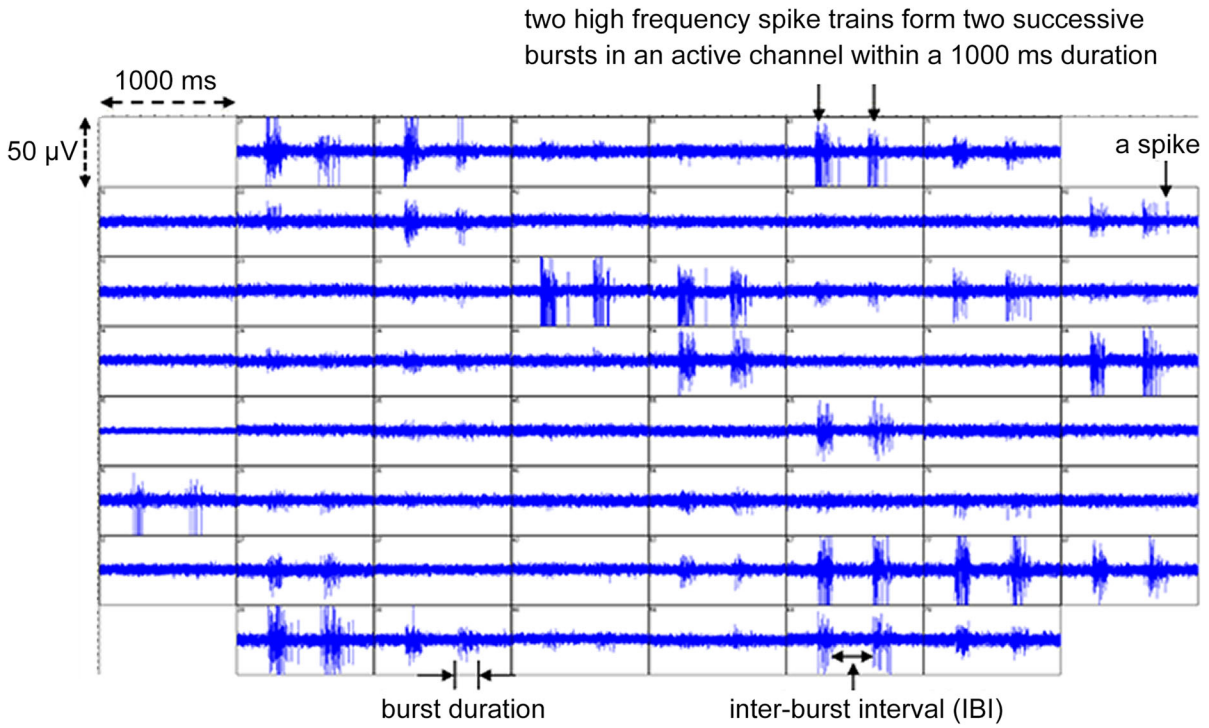

Fig. 3 Bursts on active channels organize themselves in similar patterns and occur simultaneously across most of active channels—population bursts, network-wide bursting events with synchrony in space and recurrence in time

not, and the onset of E8–E12 signals was consistent. In the E6 group, the timing of signal onset varied widely. The E15 cultures had an active channel count of less than 7 during their lifespan with some spikes or single channel bursts. However, these single-channel bursts did not synchronize to form population bursts (see the last rows in Tables 1 and 2). Since E15 neurons were more developed and differentiated, they may have experienced the greatest injury during the dissection process, so their networks could not develop well and would tend to degenerate in vitro even when supported by glial proliferation (see last row in Table 1). There could be other unknown reasons for the lack of population bursts that we have not identified.

Figure 3 shows a typical screen-shot of population bursts—network-wide, synchronized individual-channel bursts. Once a population burst occurred, it appeared recurrently throughout the lifetime of a culture. A temporal recurrence pattern is illustrated in raster plots in Fig. 6a (below).

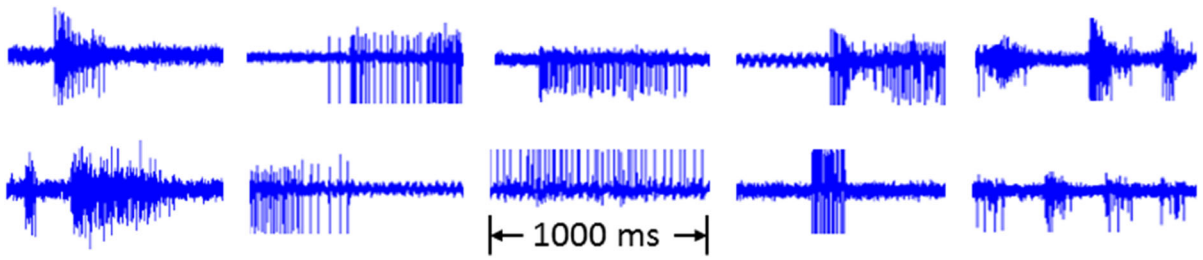

Fig. 4 Patterns of single-channel bursts and inter-burst intervals (IBI) showing the self-organization, complexity, and diversity and a degree of regularity in SSA

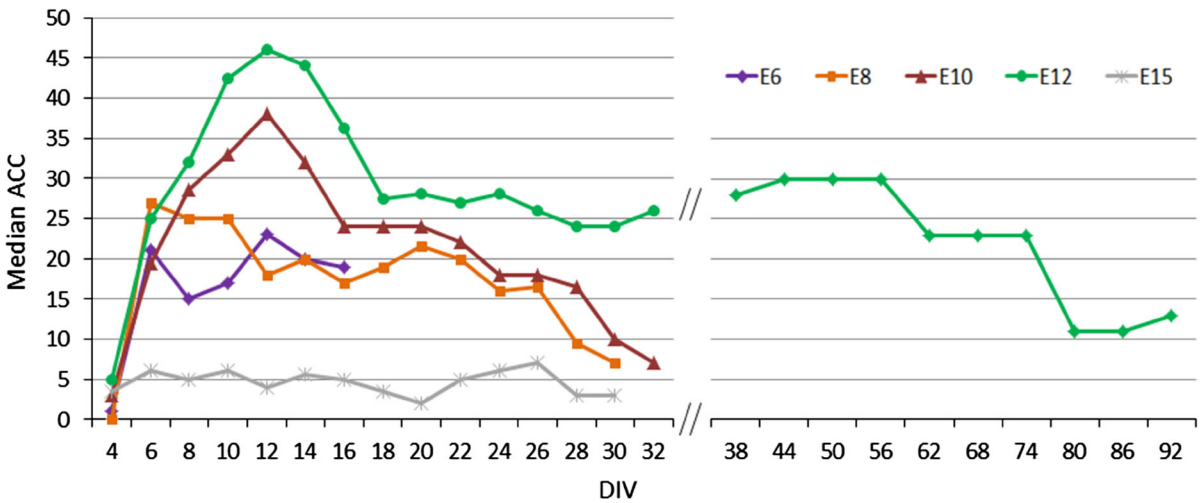

Fig. 5 Development of ACC (active channel count) across DIVs (days in vitro). Median ACC of each designated DIV was used for each embryonic age respectively. Median ACC was from 15 MEAs of 3 dissections for *E6*, 14 MEAs from 3 dissections for *E8*, 5 MEAs from 3 dissections for *E10* and *E12*, and 6 MEAs from 3 dissections for *E15*. Notice this Figure has two parts, each with a different scale at the x-axis: the first part (*left*) shows median ACC count every other day, and the second part (*right*) shows it measured every six days starting with the plateau period at about 38 DIV for *E12*

Burst organization

Wagenaar et al. (2006) described burst organization during the development of rat cortical cultures as "an extremely rich repertoire of bursting patterns". We found that the temporal and spatial features of burst organization (size, shape, duration, spike frequency within a burst, mean interspike interval within a burst, etc.) from chick FBNs were also quite diverse. Figure 4 presents the variety of burst types that appeared on individual channels from different MEAs during a time period of 1,000 ms.

Development of active channels

The absolute number of active channels (active channel count, ACC) on a particular day varied among cultures from the same dissection and varied more among cultures from different dissections. However, the overall pattern of ACC development along DIV is similar among different dissections for each embryonic age except E6. ACC development for E6 cultures varied greatly between cultures from the same dissection, and E6 cultures often detached from the MEA surface before 18 DIV. E15 cultures developed very few (1–7) active channels with some spikes, and these spikes rarely organized to form bursts, so no network-level population bursts developed. E8 to E12 cultures developed many active channels with similar patterns among different dissections. Therefore, our analysis concentrated on the E8–E12 cultures in which the ACC in general increased steeply between 4 and 5 DIV and 7–9 DIV, reached its maximum during 9–14 DIV

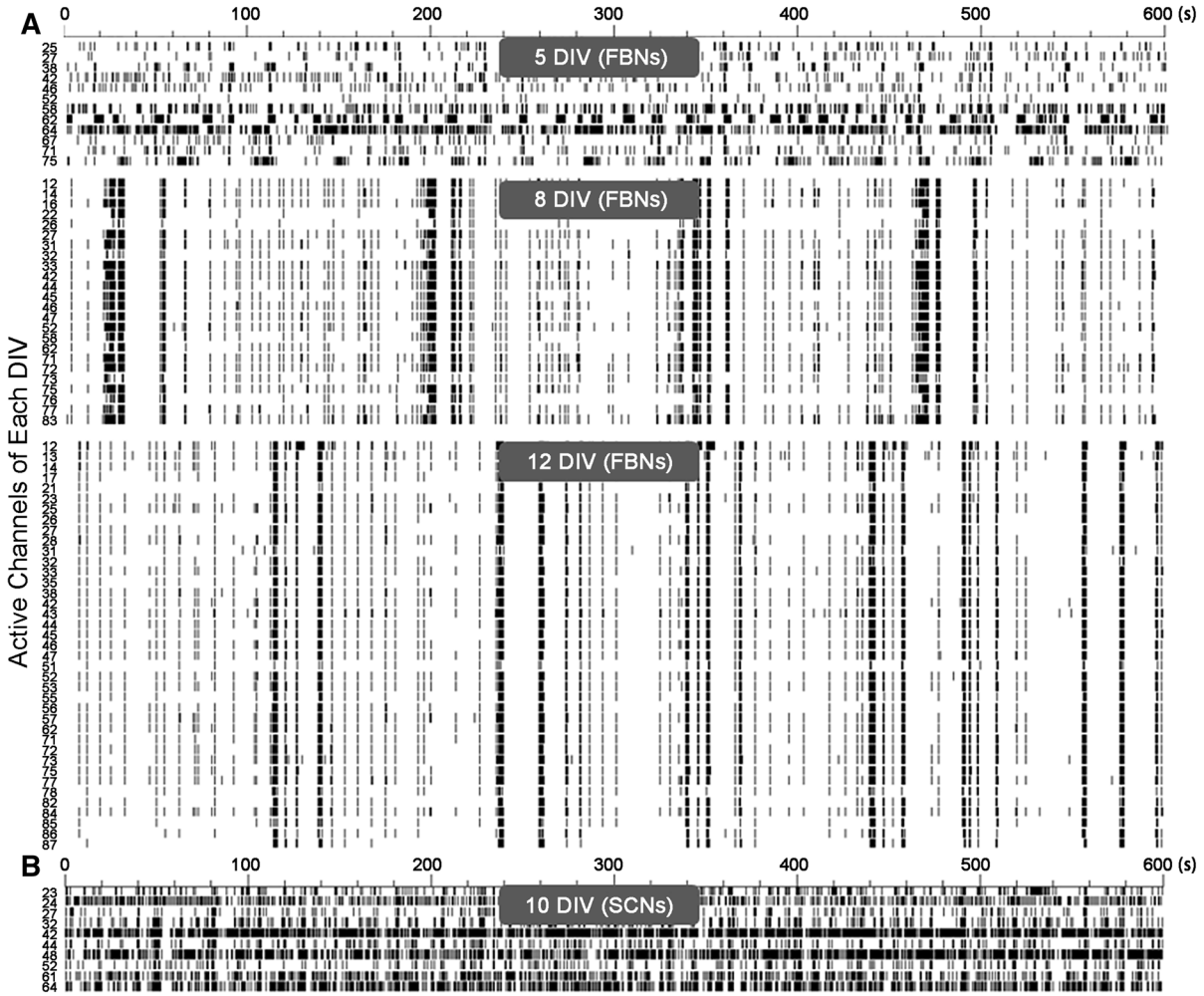

Fig. 6 Raster plots of all spikes during a 600-s recording period from **a** a spontaneous active chick FBN network and **b** a SCN network. Each *row* represents an active channel. Each *vertical bar* represents a single spike depicted at its time stamp regardless of the amplitude of the spike. **a** Raster plots at *5*, *8*, and *12 DIV* from an E10 chick FBN culture showing development and evolution of a phasic pattern of SSA firing and development of an ACC. **b** Raster plot from a chick SCN culture at 10 DIV showing a much faster firing rhythm

depending on the dissection batch and then fluctuated around a plateau. For E8 and E10, the plateau lasted about 1–2 weeks before degeneration. For E12, it lasted more than 1 month. Figure 5 illustrates the described pattern of ACC development using different-colored bars for each embryonic age. It is not clear whether the level-off of ACC is due to a possible pruning of synapses similar to that which occurs in vivo. ACC development can also be observed by an increase in the number of rows in raster plots (Fig. 6a). It is obvious that ACC development is embryonic age-dependent.

Temporal features of population bursts

A raster plot of the time stamps of each spike during a period of recording illustrates the patterns of SSA firing. Rodent cortical-neuron cultures fire in a phasic, or oscillatory, pattern of population bursts; rodent spinal cord neuron cultures exhibit a much faster rhythm (Johnstone et al. 2010). We found that chick FBN cultures also fire phasically. Figure 6a shows three raster plots for an E10 culture during a 10-minute recording at 5, 8, and 12 DIV, respectively. Active channels increased rapidly during this period as

represented by an increase in rows with DIV (Fig. 6a). A typical oscillation of population bursts was seen at 8 DIV; it evolved into 12 DIV. Figure 6b shows a raster plot from a chick SCN culture at 10 DIV. A comparison between Fig. 6a, b shows that chick FBN cultures fire in a tissue-specific pattern, like their rodent counterparts.

Discussion

We demonstrate that: 1) morphological features of FBN culture are embryonic-age dependent; 2) the in vitro lifespan of embryonic chick FBNs can be prolonged significantly and is embryonic age-dependent from E6 to E12; and 3) these cells form spontaneous active networks and have vigorous SSA that lasts throughout their lifetime in vitro. These results are of significance to the development of a chick FBN biosensor on an MEA, to a broad spectrum of other research on a neuronal network, and to investigations of comparative electrophysiology between neuronal networks developed from dissociated cortical neurons from rodent cortex and avian forebrain.

The development of MEA-based chick FBN biosensor

Our neuronal cultures produced from E6 and E8 dissections had very few glia during the first week in culture, but both were able to develop SSA during this week, indicating that the onset and early evolution of SSA were not glial cell-dependent. Cultures from E10 and E12 dissections had better culture integrity, achieved longer lifetimes, and developed more ACC than E6 and E8 samples, suggesting that glia support and maintain cultures in vitro. Therefore, the embryonic age-dependence of morphological features of chick FBN cultures will not only allow selection of forebrains with different initial neuron-glia ratios for biosensor development, it will also provide culture models for research on glia and neuron-glia interaction. For biosensor development, if the sensor will be used only once or for a very short period, cells from E8–E12 could be used; if biosensor reusability is desirable, E12 would be the best choice when M199 with 10 % FBS are used. Further characterization of the time-course of glial development in vitro would provide additional information for embryonic-age selection for different purposes of research.

Figure 6a shows that the population bursts of chick FBN cultures fire in a distinct phasic pattern that is similar to that obtained in rodent cortical-neuron cultures, but different from that obtained in a chick SCN culture (Fig. 6b). The results depicted in Figs. 3, 4, and 6a demonstrate that chick FBN SSA resembles that of its rodent counterpart: (1) at the channel level, it is self-organizing, has diverse patterns of organization, and is rhythmic; (2) at the network level, it shows a high level of synchrony of patterned events in individual channels and a tissue-specific temporal feature of phasic oscillation. The observed tissue-specificity of the firing patterns in chick FBN cultures and SCN cultures is similar to that of rodent counterparts in general. More detailed SSA analysis is needed for further comparison of similarities and differences between the two species.

A new platform for a broad spectrum of research on a neuronal network

Successful prolongation of lifetime and SSA acquisition using MEAs not only forms a new platform for biosensor development, but also enables a broad spectrum of research that can be conducted on an in vitro neuronal network with the advantages of abundant resources, cost-effective cultures, and an easy dissection process. These could include basic neuroscience studies, electrophysiological studies, synapse development and function, glial development and neuron-glia interactions, and pharmacological/toxicological studies. For example, using this platform, our results advanced in vitro chick-FBN synapse research in two aspects of its historical context (noted in the Introduction). (1) Although Tokioka et al. (1993) recorded both EPSPs and IPSPs from chick FBN cultures, they were unable to show whether the recorded EPSPs trigger action potentials in the post-synaptic neurons and whether the possible synaptic transmission occurred network-wide or sporadically in a small portion of neurons.

Here, we used MEA technology and answered both questions: (1) Population bursts—the synchrony of burst events in individual channels (Fig. 3)—indicated highly coordinated, successful network-wide synaptic transmissions; (2) the timing of the onset of spikes and population bursts (Table 2) and the early,

rapidly rising phase of ACC development (Fig. 5) in our findings corresponded very well with the findings of Louis et al. (1981) regarding morphological formation of the synapses in vitro. This indicates that synapses function soon after their formation in vitro. In contrast, Louis et al. (1981) reported that others had found that the synapse structure development in vivo was a gradual process that required many days.

A new in vitro functional tool for phylogeny and comparative biology

The six-layer neocortex is thought to be a uniquely mammalian cortical structure. Whether the structure or cell types homologous to mammalian neocortex exist in avian forebrains has long been debated (Striedter 2005). Dugas-Ford et al. (2012) have showed that neocortical cell types are conserved from reptiles to mammals and that these cells are organized into very different architectures in different species, forming cortical areas in reptiles, nuclei in birds, and cortical layers in mammals. A comparative study of the tissue-specific firing patterns of rodent and avian cortical neuronal networks that have similarity in their cell types may provide a new, convenient method for research in phylogeny and comparative biology. More detailed analysis and comparisons are needed to learn the differences and additional similarities in SSA patterns between mammalian cortical neurons and chick FBNs.

Practical applications of our findings

The successful prolongation of life and acquisition of spontaneous spiking activity signal from chick forebrain neurons in culture establish a novel and economical platform for a wide spectrum of research. This could include biosensor development for large-scale screening of potential neurotoxins, basic neuroscience studies, electrophysiological studies, synapse development and function, glial development and neuron-glia interactions, and pharmacological/toxicological studies. This functional platform can be used immediately and holds great potential for future studies with different purposes.

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Conflict of interest The authors declare no commercial or financial conflict of interest.

References

Chiappalone M, Vato A, Tedesco MB, Marcoli M, Davide F, Martinoia S (2003) Networks of neurons coupled to microelectrode arrays: a neuronal sensory system for pharmacological applications. Biosens Bioelectron 18:627–634

Dugas-Ford J, Rowell JJ, Ragsdale CW (2012) Cell-type homologies and the origins of the neocortex. Proc Natl Acad Sci 109:16974–16979

Goldman LR, Koduru S (2000) Chemicals in the environment and developmental toxicity to children: a public health and policy perspective. Environ Health Persp 108(Suppl 3):443–448

Heidemann SR, Reynolds M, Ngo K, Lamoureux P (2003) The culture of chick forebrain neurons. Method Cell Biol 71:51–65

Johnstone AF, Gross GW, Weiss DG, Schroeder OH, Gramowski A, Shafer TJ (2010) Microelectrode arrays: a physiologically based neurotoxicity testing platform for the 21st century. Neurotoxicology 31:331–350

Kandel ER, Schwartz JH, Jessell TM (2000) Part I: The neurobiology of behavior. In: Principles of neural science. McGraw-Hill, New York

Kuhn TB (2003) Growing and working with spinal motor neurons. Method Cell Biol 71:67–87

Laurenza I, Pallocca G, Mennecozzi M, Scelfo B, Pamies D, Bal-Price A (2013) A human pluripotent carcinoma stem cell-based model for in vitro developmental neurotoxicity testing: effects of methylmercury, lead and aluminum evaluated by gene expression studies. Int J Dev Neurosci 31:679–691

Louis J, Pettmann B, Courageot J, Rumigny J, Mandel P, Sensenbrenner M (1981) Developmental changes in cultured neurones from chick embryo cerebral hemispheres. Exp Brain Res 42:63–72

Pasquale V, Massobrio P, Bologna LL, Chiappalone M, Martinoia S (2008) Self-organization and neuronal avalanches in networks of dissociated cortical neurons. Neuroscience 153:1354–1369

Pirlo RK (2009) Creation of defined single cell resolution neuronal circuits on microelectrode arrays. PhD Dissertation, Clemson University, South Carolina, USA

Potter SM, DeMarse TB (2001) A new approach to neural cell culture for long-term studies. J Neurosci Meth 110:17–24

Striedter GF (2005) Principles of brain evolution. Sinauer, Sunderland

Tokioka R, Matsuo A, Kioyosue K, Kasai M, Taguchi T (1993) Synapse formation in dissociated cell cultures of embryonic chick cerebral neurons. Dev Brain Res 74:146–150

Tsai HM, Garber BB, Larramendi LM (1981) 3H-thymidine autoradiographic analysis of telencephalic histogenesis in the chick embryo: I neuronal birthdates of telencephalic compartments in situ. J Comp Neurol 198:275–292

Wagenaar DA, Pine J, Potter SM (2006) An extremely rich repertoire of bursting patterns during the development of cortical cultures. BMC Neurosci 7:11

Wigle DT, Arbuckle TE, Walker M, Wade MG, Liu S, Krewski D (2007) Environmental hazards: evidence for effects on child health. J Toxicol Environ Health B Crit Rev 10:3–39

Ylä-Outinen L, Heikkilä J, Skottman H, Suuronen R, Aänismaa R, Narkilahti S (2010) Human cell-based micro electrode array platform for studying neurotoxicity. Front Neuroeng 3