INTRODUCTION

When cultured on a microelectrode array (MEA), dissociated animal neurons form a neuronal network that spontaneously fires or spikes (Johnstone et al., 2010; Kuang, Huang, et al., 2015). This spontaneous spiking activity (SSA) is sensitive to environmental changes (physical, chemical or biological). The underlying MEA records these changes, and the coupling of a neuronal culture with an MEA forms an MEA-based neuron biosensor. Hence, a chick or a rodent spinal cord neuron biosensor can be assembled by culturing the dissociated spinal cord neurons on an MEA. Similarly, by culturing chick or rodent dissociated cortical neurons on an MEA, a corresponding cortical neuron biosensor forms. Microelectrode array-based animal neuron biosensors have been considered ‘a physiologically based...’

Abstract

A growing number of research papers report similarities in cell types, neuronal connections and information-processing principles between chick and rodent cortical tissues, which have very different architectures. This paper extends these comparisons beyond the cortical tissues. Using microelectrode array technology, we report three remarkable functional similarities between our original chick data and rodent data from the literature: (a) the pattern of spontaneous spiking activity from chick spinal cord neuron biosensors is very similar to that of rodent spinal cord neuron biosensors (i.e. rodent counterparts). (b) The spontaneous spiking activity pattern of the chick forebrain neuron biosensors is very similar to that of the rodent cortical neuron biosensors, but chick forebrain neuron biosensors contain not only cortical neurons, but also diencephalic neurons. In other words, chick forebrain neuron biosensors cannot be considered the counterparts of rodent cortical neuron biosensors. (c) Chick forebrain neuron biosensors respond to several classical neuroactive agents in a way similar to rodent cortical neuron biosensors as reflected in their agent-specific concentration–response curves and their values of EC_{50} (the effective concentration that causes 50% of the maximal effect of an agent). These preliminary findings provide both direct and indirect support for a positive answer to the big research question in the title: ‘Do chick and rodent neuron biosensors function similarly’ if the sources of the neurons are homologous between chick and rodent? Our findings are of particular value to comparative biology/physiology, pharmacology, neurotoxicology and bioengineering and to research on the more cost-effective extended application of chick neuron biosensors.

KEYWORDS

chick neurons, comparative biology/physiology, concentration–response curve, microelectrode array, spontaneous spiking activity
neurotoxicity testing platform for the 21st century’ (Johnstone et al., 2010, p. 331). Chicks (birds) and rodents (mammals) are different species, but a literature search and a retrospective comparison of our original chick data reported in this paper along with rodent data in the literature prompt the research question: ‘Are there physiological and pharmacological similarities between neuron biosensors if the sources of neurons are homologous between chick and rodent?’ Below, we discuss the rationales from the literature that stimulated this research question.

Evolution has shaped the remarkable differences in the architectures between the mammalian cortex and the avian cortex, adapting them to the needs of each species. The six-layered cortical structure of the neocortex is mammal-specific. Different mammals share a common or canonical microcircuit in their neocortex that governs information flow among layers (Calabrese & Woolley, 2015). Unlike the mammalian layered cortex, the avian cortex is organized into nuclei (Dugas-Ford, Rowell, & Ragsdale, 2012). Knowing the remarkable in vivo difference in the architectures, the research question is the following: When the neurons in the chick cortex and the rodent cortex are dissociated and cultured in vitro, whether the remarkable architectural difference no longer exists in the formed in vitro networks, might these two types of neuronal culture have more similarities than differences in their functional aspects (such as electrophysiology and pharmacology)? It depends on whether any homology (structure or cell type) to mammalian neocortex exist in avian forebrains, an ongoing debate in neocortex search (Dugas-Ford et al., 2012; Reiner, 2013; Wang, Brozowska-Prechtl, & Karten, 2010).

The argument of cell type homologies is supported by three recent publications in PNAS. Wang et al. (2010) found remarkable anatomical similarity in neuronal connectivity patterns between the cortices of birds and mammals. Dugas-Ford et al. (2012) used a molecular approach based on layer-specific gene expression, to confirm that neocortical layer 4 input neuron type and layer 5 output neuron type are conserved from reptiles to mammals. These cells are organized into very different architectures in different species, forming cortical areas in reptiles, nuclei in birds and cortical layers in mammals. Calabrese and Woolley (2015) used in vivo microelectrodes to record the activities of single neurons and population neurons; they showed that ‘the same information-processing principles that define the canonical cortical microcircuit, long thought to have evolved only in mammals’ are found in the avian cortex, which suggests ‘that the canonical cortical microcircuit evolved in a common ancestor of mammals and birds and provides a physiological explanation for the evolution of neural processes that give rise to complex behaviour in the absence of cortical lamination.’ (Calabrese & Woolley, 2015, p. 3517).

In addition, dissociated rodent neurons reportedly have retained similar morphological and physiological in vivo phenotypes and similarity in in vivo activity-dependent path-specific synaptic modifications (Bi & Poo, 1999; Jimbo, Tateno, & Robinson, 1999; Kriegstein & Dichter, 1983). Some researchers speculate that a cultured neuronal network is a simplified and accessible model of the central nervous system (Scelfo et al., 2012) that can be used as a biosensor to detect neurotoxic chemicals in the environment.

The above findings suggest that some cortical tissue-specific synaptic connections or microcircuits are retained in vitro neuronal networks reformed from dissociated cortical neurons. Logically, the following reasoning should stand: when neurons dissociated from the cortical tissues of birds and mammals are dissociated and cultured in vitro, the remarkable difference between avian and mammalian in vivo architecture no longer exists. After these dissociated neurons reform neuronal networks in cultures, it is reasonable to hypothesize that more similarities than differences in their functional aspects (electrophysiology and pharmacology) should be seen between the two species. Hence, if chick cortical neuron biosensors (C-CN-biosensors) share physiological and pharmacological similarities with rodent cortical neuron biosensors (R-CN-biosensors), chick spinal cord neuron biosensors (C-SCN biosensors) should also be more likely to share physiological and pharmacological similarities with rodent spinal cord neuron biosensors (R-SCN biosensors) because the architectural differences between chick and rodent spinal cords are less remarkable than those between chick and rodent cortices.

In terms of the reasoning above, in our development of the chick neuron biosensors in the past (Kuang, Wang, et al., 2015) and in this study, we made C-SCN-biosensors. However, we did not make C-CN-biosensors. We made chick forebrain neuron biosensors (C-FBN-biosensors). Chick forebrain includes the telencephalon and diencephalon (Jessell & Sanes, 2013). The pallium in the chick forebrain is the homolog of the mammalian cortex (Dugas-Ford et al., 2012; Jarvis et al., 2005; Olkowski et al., 2016). Thus, the chick pallium can be considered the counterpart of the mammalian cortex, whereas the chick forebrain is more than the cortex.

Using our C-SCN-biosensors and C-FBN-biosensors, we report here three original findings: (a) the SSA pattern (i.e. the natural electrophysiological behaviour) of the C-SCN-biosensors obtained in this study is very similar to the SSA pattern of the R-SCN-biosensors in the literature; this supports a positive answer to our research question. (b) The SSA pattern from the C-FBN-biosensors obtained in this study is very similar to the SSA pattern of the R-CN-biosensors in the literature; this indirectly supports our research question. (c) Pharmacologically, the C-FBN biosensors in this study responded to several classical neuroactive agents in a way similar to the R-CN-biosensors in the literature; this indirectly supports a positive answer to the research question because chick-FBN biosensors contain cortical neurons.

Since animal neuron biosensors can be used as a functional platform to conduct research studies in multiple disciplines (comparative biology/physiology, pharmacology, neuroscience, neurotoxicology, etc.) and for different research purposes (e.g. neuron biosensor development and application), our research findings, though limited, are of value to draw the attention of researchers in these various disciplines.
2. MATERIALS AND METHODS

2.1 Cell culture

The procedures for dissection and dissociation of the embryonic day 9 (E9) chick forebrain and spinal cord followed Heidemann, Reynolds, Ngo, and Lamoureux (2003) and Kuhn (2003), respectively. Dissociated FBNs and SCNs were cultured on the MEAs in the same way as reported in our previous papers (Kuang, Huang, et al., 2015; Kuang, Wang, et al., 2015). The procedure for using chick embryos (E9) was 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 (2011).

2.2 MEA recording and spike detection

Spontaneous spiking activity signals from both C-FBN-biosensors and C-SCN-biosensors were recorded in the same way (Figure 1a), as described in our previous publication (Kuang, Huang, et al., 2015). MC_Rack software extracted spike information simultaneously. A spike is the extracellular measure of the action potential of a neuron.

2.3 Burst detection and raster plot

A burst is a self-organized high-frequency train of spikes (Figure 1b). Bursts were detected using NeuroMEA, a MatLab-based program we developed and defined using the three criteria described in our previous papers (Kuang, Huang, et al., 2015; Kuang, Wang, et al., 2015). NeuroMEA also created raster plots, which are commonly used with MEA data to present temporal and spatial features of SSA. To create a raster plot, NeuroMEA allows a user to select active channels and a time window within which to show MEA signals. NeuroMEA then aligns the selected active channels in rows and plots the time stamp of each spike that appears on each selected active channel within a predesignated time window. The alignment of rows of active channels and the plots of the time stamps of each spike during the designated time window on each active channel form a raster plot (Figure 2). This allows a researcher to examine the temporal features of SSA (the mean burst rate (MBR) and the rhythmicity of SSA in this article) and spatial features of SSA (the synchronicity of bursts net-wide) directly.

Like the methods used with R-CN cultures in the literature, C-FBN-cultures were allowed 3 weeks to fully develop their synaptic connections without exposure to any neuroactive agents in this study. During these 3 weeks, most spikes tended to be packed into bursts progressively. After 3 weeks (4 weeks for cultures with less cell density), depending on the cell density in a culture, more than 90%-95% were packed into bursts, and this ratio remained relatively stable for months before the culture degenerated. Bursts in different active channels fired in a synchronized pattern recurrently. These spontaneous, well-coordinated, net-wide recurrent burst events are called population bursts.

2.4 Neuroactive agents

Using concentration–response experiments, the C-FBN-cultures were tested for their responsiveness to two inhibitory neuroactive agents: the voltage-gated sodium channel blocker tetrodotoxin (TTX, 1069-1 MG, Tocris Bioscience) and the glutamate N-methyl-D-aspartate (NMDA) receptor blocker, magnesium ion or Mg2+ (MgCl2.6H2O, M2670, Sigma).

2.5 Concentration–response experiment and curve fitting

Two-step curve fitting has been described in a previous publication (Kuang et al., 2016). In brief, step 1 is to use NeuroMEA to plot a concentration–response (x-y) curve for each concentration–response experiment. The starting point of the curve is the MBR of the baseline value averaged from all active channels from one biosensor. This
baseline value is the reference used as a control when no dose was administered. This control value of MBR is set at 1 (100%) on the y-axis. The MBR at each concentration was calculated in the same way and was normalized to be a percentage of the control value. For each agent, four concentration–response curves were obtained by repeating the experiments using four biosensors.
In step 2, these curves were finally fitted into one sigmoidal concentration–response curve for each agent based on the Hill equation (Novellino et al., 2011; Weiss, 2011) using GraphPad Prism 5 software:

\[ y = \frac{y_{\text{START}} + (y_{\text{END}} - y_{\text{START}})}{1 + 10^{\left(\log(y_{\text{END}}) - \log(y_{\text{START}})\right) - \HC}} \]

where \( y \) is the observed value of a response, \( y_{\text{START}} \) is the highest observed value, \( y_{\text{END}} \) is the lowest observed value at the highest dose, \( EC_{50} \) is the effective concentration that causes 50% of the maximal inhibition, and \( HC \) is the Hill coefficient (the slope at the inflection point of the curve, the largest absolute value of the slope of the curve).

3 | RESULTS AND DISCUSSION

3.1 | The SSA patterns from our C-SCN-biosensors in this study and the R-SCN-biosensors in the literature are very similar

Figure 2a shows the tissue specificity of the SSA patterns of both the R-SCN-biosensors (left) and R-CN-biosensors (right) from the literature (Johnstone et al., 2010). In Figure 2b, we aligned our original findings from the C-SCN-biosensors (left) and the C-FBN-biosensors (right) in the same way using the same time scale as in Figure 2a.

Obviously, the SSA patterns of the C-SCN-biosensors and their rodent counterparts (i.e. the R-SCN-biosensors) are strikingly similar. This comparison directly supports that the physiological outputs or behaviours (as reflected in the SSA) of the SCN-biosensors of both species share striking similarity. It should be the spinal cord tissue-specific synaptic connections or the microcircuits and the electrophysiological properties of the spinal cord neurons (such as their levels of threshold potentials and their refractory periods) that together determine the SSA pattern for both C-SCN-biosensors and R-SCN-biosensors. Hence, this result further suggests that the spinal cord tissue-specific synaptic connections or microcircuits are conserved from birds to mammals.

3.2 | The SSA patterns from our C-FBN-biosensors in this study and the R-CN-biosensors in the literature are very similar

If we compare the right halves of Figure 2a,b in this study and if readers see the many raster plots from different C-FBN-biosensors at different times of their lifespan published in our previous paper (Kuang, Huang, et al., 2015), the SSA patterns of the C-FBN-biosensors and R-CN-biosensors will be seen to be very similar and both exhibit a phasic pattern. It is not possible to distinguish one from the other if they are not labelled. Why do C-FBN-biosensors that contain diencephalic neurons exhibit an SSA pattern similar to the R-CN-biosensors? One possible answer is that evolutionally, the telencephalic pallium (i.e. avian cortex), like its rodent counterpart (i.e. rodent cortex), has evolved to be the most advanced brain structure of birds and thus dominates the SSA pattern in the C-FBN-biosensors. If this explanation is true, it is less likely that the SSA pattern of the chick pallial neuronal cultures is not similar to that of the rodent counterparts, R-CN-biosensors.

A second explanation is that patterned spatial–temporal firing is seen in the neuronal networks of invertebrates (Ayali et al., 2004), and it is assumed that ‘fundamental principles would probably be more conspicuous in a simple invertebrate nervous system. These principles may however be relevant to all systems generating synchronized bursting activity’ (Fuchs, Ayali, Robinson, Hulata, & Ben-Jacob, 2007, p. 1812). Hence, it is reasonable to consider that both chick forebrain and spinal cord evolved from the more fundamental neuronal organization and share the fundamental firing principles. Since the diencephalon is close to the telencephalon and the spinal cord is far from the telencephalon, the diencephalic neurons in the C-FBN-biosensors are likely to exhibit an SSA pattern similar to that of the pallial neurons. Future research studies should compare the SSA patterns of biosensors made of pallial neurons, diencephalic neurons and entire FBNs between the two species to discover their similarities and differences.

3.3 | The SSA responsiveness of C-FBN-biosensors towards selected classical neuroactive agents in this study is similar to that of R-CN-biosensors in the literature

Figure 3 presents the sigmoidal concentration–response curve fitting for TTX and Mg²⁺ from this study. In Figure 3a, a final sigmoidal curve for TTX (left) was fitted from four individual curves (right) obtained from four concentration–response experiments conducted on four different C-FBN-biosensors. In Figure 3b, a final sigmoidal curve for Mg²⁺ (left) was fitted in the same way from four individual curves (right). The blue arrows in the two fitted curves indicate the concentration of \( EC_{50} \) for TTX and Mg²⁺, and the GraphPad Prism 5 output of the values of \( EC_{50} \) for TTX and Mg²⁺ was 9.8 nM and 563 μM, respectively (see also in Table 1).
In Table 1 below, we compare our original data of the estimated values of EC_{50} of TTX and Mg^{2+} obtained from C-FBN-biosensors in this study with those from R-CN-biosensors in the literature. These pharmacological results indicate that the SSA of C-FBN-biosensors responded to selected neuroactive agents in the same concentration-dependent pattern within the same or similar log_{10}[concentration] ranges as those of the R-CN-biosensors. The values of the estimated EC_{50} for these agents fall into the same orders of magnitude as those for the R-CN-biosensors. These results also suggest that the C-CN-biosensors and the C-SCN-biosensors are likely to respond to these four neuroactive agents in ways similar to the R-CN-biosensors and R-SCN-biosensors, respectively.

In addition, our results in one of our previous papers (Kuang et al., 2016) also support this statement; that is, we obtained values of EC_{50} of AP5 and muscimol from our C-FBN-biosensors that were similar to the values obtained from the R-CN-biosensors in the literature.

### Table 1: Comparison of the concentration ranges used for the concentration–response experiments and estimated values of EC_{50} obtained from C-FBN-biosensors for TTX and Mg^{2+} (newly presented in this paper) with the corresponding information of R-CN-biosensors from the literature

| Agent | Concentration range (Chick) | EC_{50} (Chick) Mean ± SE | Concentration range (Rodent) | EC_{50} Rodent Mean ± SD or SE | Type of rodent | Reference |
|-------|-----------------------------|---------------------------|-------------------------------|-------------------------------|----------------|-----------|
| TTX   | 0.1–30 nM                   | 9.81 ± 1.40 nM            | 0.1–100 nM                    | 1.1 ± 0.2 nM (SD)             | Rats           | Otto et al. (2003) |
| Mg^{2+}| 10 μM–10 mM                 | 563 ± 290 μM              | 0.1 μM–10 mM                  | 161 ± 23 μM (SD)              | Rats           | Otto et al. (2003) |

### 3.4 General implications of the above results

First, in our experimental setting, we did not see observable physiological and pharmacological differences between C-SCN-biosensors and their rodent counterparts, nor between C-FBN-biosensors and R-CN-biosensors. Therefore, the results reported
above, although preliminary, may partially answer the research question reflected in the title of this article. To further test the research question, not only chick pallial neurons but also neurons from other functional regions of the chick and rodent brains (such as the cerebellum) need to be cultured on MEAs and compared. To detect the possible differences, more refined research studies need to be designed and conducted.

Second, because of the remarkable similarities between the SSA patterns of the C-SCN-biosensors and their rodent counterparts, many foundational biomedical research studies (biological, physiological, pharmacological, toxicological, etc.) and bioengineering research studies that use rodent spinal cords may be conducted using chick spinal cords. It is also possible to use chick pallium neuronal cultures instead of using rodent cortical neuronal cultures to estimate the E50 of other neuroactive agents or the neurotoxic chemicals in the environment. This approach would be much more cost-effective than using rodent neurons for large-scale, screen-based detection of the environmental neurotoxic chemical compounds that pollute the environment.

Third, the SSA of a neuronal culture is its natural physiological behaviour. A sigmoidal concentration–response curve shows how a biological sample responds to a specific ligand (pharmacological behaviour), suggesting that the receptors of the ligand (i.e. the receptors of TTX and Mg2+ in this study) are expressed and function in the biological sample.

Fourth, two of our past studies (Kuang, Huang, et al., 2015; Kuang, Wang, et al., 2015) have shown that chick and rodent neuron biosensors also share similarities in their developmental and metabolic aspects: it was difficult for E6 to E10 C-FBN-biosensors to form confluent cell monolayers in Medium 199, and they had a lifespan of only about 1 month (Kuang, Wang, et al., 2015). However, they thrived in the Neural Basal cell culture medium, which was designed specifically to culture rodent cortical neurons (Brewer, 1995; Brewer, Torricelli, Eveyge, & Price, 1993). They formed confluent cell monolayers in about 1 week and had a lifespan of several months, which was also similar to the R-CN-biosensors.

3.5 | Limitations of the study

First, our comparison with rodent results from the literature is not ideal, which may confound the validity of our comparisons. However, this seems not to be a critical concern because the similarities we found are remarkable. This suggests that if chick and rodent cortical neuron biosensors are developed in the same experimental setting and in the same laboratory, it is reasonable to expect to see more striking similarities in physiology if the neurons are dissociated from the homologous regions of chick and rodent. If they are developed in the same laboratory, it would be easier to discern the possible differences between the two species in physiology and pharmacology that we have failed to discover to date.

Second, the values of EC50 we obtained were rough estimations. To obtain an accurate value of EC50 for each agent with C-FBN-biosensors, future concentration–response experiments should put more focus on the time range of the linear portion of each fitted sigmoidal curve and add more concentration points. Again, if neurons are dissociated from the homologous brain regions of the two species and if the neuron biosensors are developed in the same laboratory, more pharmacological similarities should be seen between the two species.

Third, the comparison made in this paper was preliminary. Future researchers are advised to access publicly available rodent MEA datasets and conduct quantitative and statistical comparisons with a large sample size.

AUTHOR CONTRIBUTIONS

Serena Y. Kuang proposed the research question, cultured cells, did about half of the concentration–response experiments and wrote the manuscript. Xiaqi Yang conducted half of the concentration–response experiments, processed all the data, developed all the figures and participated in discussion of the manuscript. Lina Wei assisted in MEA recording, dissected and cultured the chick spinal cord neurons, and participated in discussion of the experiments. Ting Huang helped with the concentration–response experiments and assisted with the development of NeuroMEA. Zhonghai Wang was responsible for MEA technical support and programmed NeuroMEA for MEA data processing. Tingfei Xi and Bruce Z. Gao sponsored the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Bruce Z. Gao https://orcid.org/0000-0003-3476-1927

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How to cite this article: Kuang SY, Yang X, Wei L, et al. Do chick and rodent neuron biosensors function similarly?. Med Devices Sens. 2020;3:e10078. https://doi.org/10.1002/mds.3.10078