Impact of Training on Perception in Dissociated Cortical Culture Neural Networks

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Abstract

The way we recall events has an impact on how we perceive them. A good example of this is visual illusion. Can memorized stimuli influence the acquisition of a variety of stimuli in tiny neural networks? To answer this question, we used dissociated cortical culture (DCC) grown on a multielectrode array. This *in vivo-like in vitro* system allows tracking and assessing structural and functional refinement, as well as the ability for information acquisition, processing, and coding in neural networks. A range of electric stimuli were used to simulate sensory inputs and to train DCC neural circuits. Single, paired-pulse (PP, 20ms ISI) 300mV stimuli at 5, 10, 20, 50, and 100Hz for 1s were delivered in a random time interval. During the analyses, the pre-training, training and testing phases were compared.

The data revealed that registered channels increased activity in response to one of the stimulus types while responding less effectively to others. Single, 5Hz,

and notably PP stimuli were the favored paradigms. The training phase frequently showed a progressive increase in activity level, with short burst prevalence. However, repetition of the preferred stimuli enhanced the occurrence of both tonic and burst evoked responses with prolonged duration throughout the testing phase. The data demonstrate that even such a tiny network of neurons as we have in DCC is capable for selection of the sensory inputs according to previously memorized information and at their most basic levels they are efficient of performing some functions that are typically attributed to higher neural structures.

KEY WORDS: in vivo-like in vitro; dissociated cortical culture; multielectrode array; neural plasticity; sensory discrimination; neural network

Introduction

In humans and higher vertebrates, sophisticated nervous system functions like memory, sensory processing and perception necessitate the engagement of the high order brain structures. These phenomena are interweaved, and impact processes on a psychological level. Visual illusions are an excellent example of this. During visual illusion, we perceive visual stimuli differently than they are in reality. This happens because our memory affects our sensory processing and evantually our comprehension of how we see.

However, whether sensory processing and memory, characteristic feature of high order nervous system functions are present and interrelated at the level of local neural circuits is a fascinating question in neuroscience. In some modern research, it has been established that input signals have a significant influence on the formation of network features in developing neural networks [1]. In fact, how brain tissue functions is determined by the physical structure of specific circuitries [2].

To understand how sensory processing and memory interact on a local circuit level, multielectrode arrays (MEA) with simplified neural networks of dissociated cortical culture (DCC) can be used for effective scientific approaches such as stimulation and registration of multiple electrophysiological signals. DCC naked neuronal cells change

shape and acquire axons and dendrites that connect to one another during development, creating neural networks similar to those found in neural tissue. Implanted on the glass surface electrodes designed for both stimulation and recording in the parallel regime allow for the simulation of sensory inputs over various areas of neural culture on the one hand, and the recording of a variety of evoked electric neural signals from the entire surface on the other, reflecting the complexity of information processing [3]. This system becomes an effective scientific tool for modeling brain to body feedback systems [4, 5] and even prototyping neuro-prosthetic or brain to machine cyborg systems using complicated algorithms, advancing a topic of great medical significance [6, 7]. As a result, they're frequently referred to as in vivo-like in vitro system [8, 9, 10].

Previous work developed feedback cyborg systems of DCC and robotic body [6, 7, 8, 9] in such systems a neural network continuously receives information about the orienting stimuli from the robotic body, saves information, and improves performance over time, all of which dependended on neural plasticity processes in the neuronal culture. Researchers learned how to employ brain signals to operate machines using this impressive scientific approach, and simultaneously, they could investigate mechanisms of high nervous functions in a highly appropriate long-term easily viewable dynamic preparation of DCC neural networks [4, 10, 11].

Our previous work helped us in approaching the topic and provided a more conducive environment for research. Over time, the population of about 100000 neuronal and glial cells in our experimental setting formed a simplified but realistic brain structure and could live on MEA for two months. This allowed us to track and assess the structural and functional refinement of freshly formed neural circuits, as well as their capacity for information collection, processing, and coding. This helped us to see the importance of single-neuron activity in these processes. Evoked responses included both instant and later responses during the training period that lasted an indefinite period of time and varied depending on the experimental conditions. That phase frequently included a progressive increase in activity level, with burst prevalence being common. However, during the testing period, repetition of the chosen stimuli raised the background level of activity and, in particular, the occurrence of evoked responses with a prolonged effect.

The data demonstrate gradually realized synaptic plasticity mechanisms for memory formation to favored stimuli, on the one hand and the beneficial influence of the retained information for particular responses to preferred stimuli, on the other hand. As a result, we can conclude that even a small neural network of population neurons, such as the one used in DCC, is capable of selecting sensory information based on previously memorized data. This demonstrates that neural networks at their most basic levels are capable of performing complex functions that are typically attributed to higher neurological structures.

Materials and Methods

Experiments were carried out in accordance with the guidelines of the International Animal Care and Use Committee (IACUC) and the Free University of Tbilisi's bioethics committee.

Preparation of DCC

With major alterations to fit our experimental requirements, our experimental approaches were based mostly on the works of Potter and colleagues [3], which described protocols for DCC preparation, care and electrophysiological registration on a multielectrode array. Briafly, cortical slices were obtained from the brain of a rat fetus on the 18th day of pregnancy (total number 20 from the 8 litters). Sterility was ensured with the use of a biosafety cabinet class 2 and 70% alcohol. A cold Hanks balanced salt solution (HBSS) was used for placing cortical fragments and cleaning neural tissue from blood and arachnoid remains. In order to decrease excitatory processes related to seizures, excitotoxicity, and apoptosis in mechanically dissociated brain tissue, AP5 (0.025 mM final concentration), a selective antagonist of NMDA receptors and Kynurenic acid (final concentration 1 mM), antagonist of ionotropic excitatory amino acids were added to solution. A mixture of active papain (15 units) and DNase (50 M) was used for tissue fermentative digestion for 20 minutes. The digested tissue remnants were rinsed with Thermofisher's CO_2 independent hibernation medium with B27 plus supplement (2%) and fetal bovine serum (10 %). Special microfilters with 40 m phores were employed to obtain a clear colony of dissociated cells (neurons and glial cells mixed) and a clean solution from the remains. After that, bovine serum albumin was added to the solution. It was centrifuged at 200x speed and rinsed with a final medium to eliminate toxins generated during the process of tissue digestion and dissociation from the cells. Using a cytometer grid and a microscope, the cell concentration was estimated to be around 5000-6000 cells per l μl. Dilution or centrifugation were utilized to adjust for the outraging of a standard concentration. There was no attempt to separate glial cells because they aid in the survival of neurons and provide natural room for development. For 30 seconds, 15-20 μl of cell suspension was poured over the surface of MEA electrodes that had been pre-coated with polyethilen imine (0.05%) and laminin (0.001%). The cells were allowed to attach to the surface for 30 minutes before adding the final media to the MEA basin. HEPES (hydroxy-ethil-hyperazin-ethan-solphonic acid) solution (0.01 M final concentration) was added to the surrounding tissue media

for enhanced buffering qualities. The incubator's temperature was 36°C, with a humidity level of 65%. Half of the medium was usually refreshed twice a week. The other half was left to ensure a sufficient level of intrinsic cell survival factors. To maintain sterile conditions with the ability to transfer surrounding CO_2 (5 %) in the incubator, a suitable cover with teflon milliphore membrane (from multichannelsystems Co.) was enclosed to the ring of MEA (Fig. 1 A). DCC was kept alive in those conditions for two months to ensure that the tissue was healthy and capable of generating action potentials.

Morphological control

To monitor the morphological condition of DCC cells for electrophysiological investigation, an inverted digital microscope with a magnification of 200-400X was used. Morphological control was performed during cytometric adjustment of cell concentration on the day of culture preparation, and after, twice weekly before electrophysiological recording sessions. The images were taken with the digital camera's included AMCAP software. Microscopy was used to determine cell concentration, region cleanliness, cell distance from electrodes, and neural fiber growth degree. For better comparison, pictures were obtained from the same two areas across all experiments: The first was a 9-electrode area around the electrode N22, and the second was a 9-electrode area surrounding the electrode N77. The length of neural fibers was simply compared to the diameter of electrodes (30 μm) using basic geometric methods, and the degree of development of neural networks was estimated.

Electrophysiological recording

Setup of 60 channel electrophysiological systems The MEA1060-UP-BC preamplifier is designed for 60 electrode MEA recordings and includes blanking circuitry that allows registration of the stimulating electrodes with the others. The preamplifier, stimulator STG4002, and computer (with the supplied proper cardboard) are all synchronized to ensure the system's coordinated operation. The free softwares: MC_Rack, MC_Stimulus, and MEA_Select were used for registration, stimulation, and selection of the sets of stimulating and grounding electrodes (Multichannelsystems Co., Germany).

Before beginning experimental recordings, proper electric stimuli protocols were created in the MC_Stimulus software, which is designed to provide synchronously electric stimulations for MC_Rack recordings. To imitate diverse sensory inputs to DCC and to train the specific neural networks, various forms of electric stimuli were used. Rectangular impulses with two phases of 100 μs duration and voltage of 300

mV were used for single, paired-pulse (PP, with 20 ms ISI) and varied frequencies of stimulations of 1, 5, 10, 20, 50, and 100 Hz of stimuli of 1 s duration. A pair of electrodes delivered certain types of stimulations once every 20 seconds (or at a close random time interval). The length of the stimulation sessions, which were eventually divided into training and testing phases, varied depending on the experimental settings and responses.

Before stimulation sessions began, spontaneous multi-neuronal activity was always recorded as a baseline level for evoked activity. The signals were recorded at sampling rate 25 kHz and filtered using a Butterworth 2nd order high-pass filter (>200). Following that, stimulation operations were performed, followed by registration for the proper period of time. The registering software's multichannel structure, as well as the electrode array, enabled for dimensional dispersion of signal processing.

Data analyzes

For neural data analyses, MC Rack, the data collecting software, offers limited capabilities. However, the multichannel DataManager application allows to convert MCD to HDF5 format. The Python programming language was used to create a data analysis program application pack (NeuroSpace) that allowed to move data to appropriate graphical and numerical datafiles before being analyzed in the SPSS statistical program. The presentation of real-time signals from a single channel, the separation of stimulus-induced evoked frequencies, the processing of specified partitions, the separation of single-units, the detection of neuronal and network bursts, and the generation of appropriate datafiles were all possible with NeuroSpace. This approach was used to investigate up to 30 MC_Rack electrophysiological recordings in total.

Electrophysiological data was obtained from matured DCCs aged 25 to 35 days in vitro (DIV). The key categorization criteria related to the stimuli used for certain training session were: 300 mV single, PP, and varied frequencies of 1, 5, 10, 20, 50, and 100 Hz for 1 s. Each of them was used for a prolonged period and the recorded data was examined at three different levels: 1. the baseline level of activity obtained from the phase of recording before stimulations began, 2. the training phase during stimulation when neuronal activity was gradually increasing, and 3. the testing phase after stimulus evoked activity reached its maximum level. Spike frequencies of multi-unit and single-unit activity, which were determined before and after the applied stimulus paradigms, were the parameters of interest for evaluation. Three distinct methods were used to make these comparisons: Instant response (up to 300 ms) intervals; longer-term (up to 2000 ms) periods; and general activity levels elicited by specific stimuli.

Despite some meticulous planning, data showed significant variations in some cases. Thus, one of the most appropriate approach for analyzing the effect of specific stimuli patterns was to use the percentage of probability of induced responses.

Statistics

IBM SPSS statistics software was used to conduct the statistical analyses. Univariate ANOVA analysis with Bonferroni post-hoc tests were used to assess activity at different phases within the different stimulation groups. For phase and pre – vs. post-stimulus (for 2 s) comparisons, the same two-factor test was used; two-way ANOVA analyses were required to assess the impact of stimuli on the phases within the groups; multi-factorial ANOVA tests with the same Bonferroni post-hoc analysis were used to investigate the impact of different stimuli types on pre – vs. post-stimulus conditions (for 2 s) in different phases.

Results

Morphology

After adjusting the cell concentration to the appropriate range of 5000-6000 cells in 1 μl, morphological monitoring of neuronal culture began on 0 DIV. Cells were bare oval/round-shaped white bodies with no fiber descendants at the time. This demonstrated that enzymatic digestion of neural tissue resulted in the loss of the characteristics that bind cells together to form neural tissue, and we acquired dissociated healthy cells (Fig. 1, A). Morphological control was done twice a week before electrophysiological sessions during the experiments. At 3-4 DIV, differentiated cell bodies were visible, and axo-dendritic fibers reached about 42$\pm$13 μm after 7 DIV (n=30). Following weeks of cultivation, there was a gradual increase in cell fibers: 64$\pm$15 at 14 DIV, 78$\pm$23 at 21 DIV, which did not change significantly later (Fig. 1, B, C). Although some cells demonstrated fiber expansion after 3-4 weeks, there was a consistent reduction in cell number across the counted region.

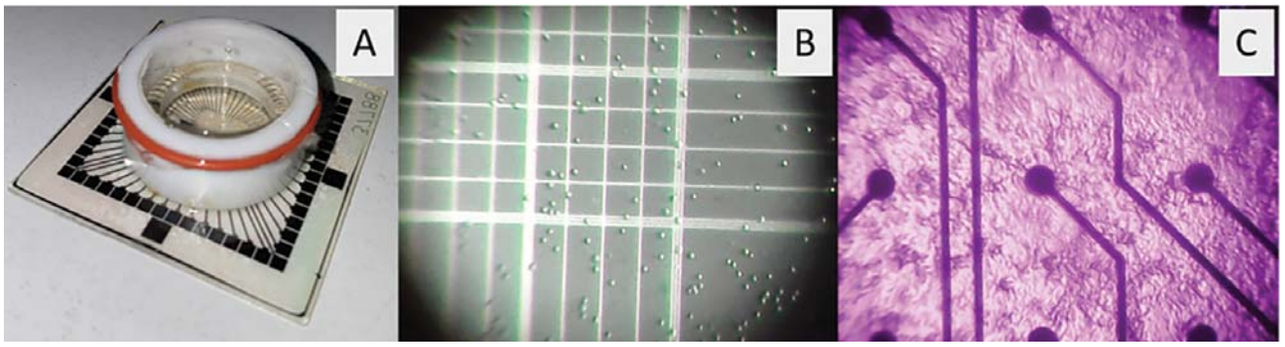

Fig. 1. *A – Multielectrode array (MEA) with Teflon Millipore cover. B – DCC on the hemocytometer at 0 DIV, exhibiting adequate dispersion of about 25 healthy cells on 0.04 mm2 (6250 cells in 1 μl) area. C – DCC attached to the MEA surface at the day 25 DIV, revealing developed neuronal network with axonal and dendritic fibers.*

Electrophysiology

The MC_Rack software, which is freely available on the multichannelsystems Co. website, was used to make electrophysiological extracellular recordings of multiunit activity. All subsequent processing was carried out using the NeuroSpace software, which we created in Python. That allowed for both graphical and statistical processing of the data obtained from MEA recordings. For this work, electrophysiological investigations were performed on 18 DCC preparations restricted in age from 25 to 35 DIV in order to prevent fluctuating changes in electrophysiological characteristics.

Before each session of stimulation, spontaneous responses were recorded for roughly 10 minutes to establish a baseline level of activity. This activity was characterized by a wide spectrum of responses, ranging from rare spikes to intense bursts that became stronger with age. Single-unit analyses revealed that most of the time bursts were due to activity of several different neurons. Despite this, single neuronal bursts were detected in some cases as well. The channel had to be active for long enough to register for both spontaneous and stimulation sessions, which was a necessary prerequisite for registration (at least 30 min). Random pair of electrodes were utilized to induce a range of electric shock sessions using random time intervals after a spontaneous activity registration sessions.

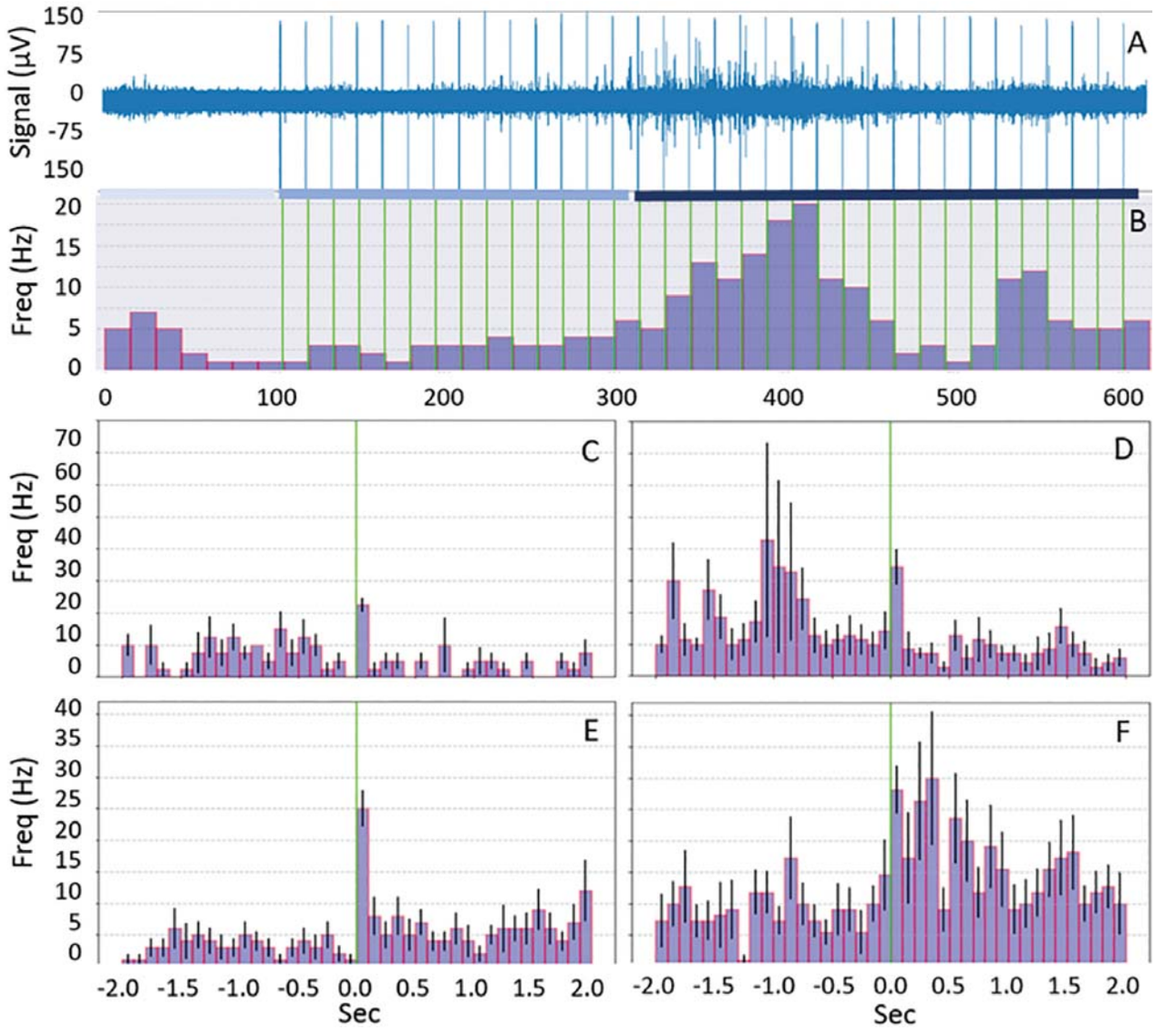

Fig. 2*. Neural plasticity-dependent modifications in multi-unit neuronal responses to continuous PP electric stimulation. The background, training, and testing phases of the recorded activity are indicated by colored underlines. A – Spontaneous activity followed by 34 PP stimuli that elicited steadily increasing responses during training phase and stable evoked responses during testing phase. B – Frequency diagram of the same recording illustrating dynamic changes between training and testing phases. C, D – Burst-dependent inhibition with PP stimuli displayed separately, with pre-stimuli and post-stimuli of 2 s intervals summarized. E, F – During the training (E) and testing (F) phases, a frequency diagram of the summed pre – and post-stimulus activity of 2 s intervals demonstrates persistent responsiveness to the same stimuli.*

As it was previously reported by us, DCC neural networks reveal capability to dscriminate variety of sensory inputs and respond specifically to the provided stimulations. For this research, we applied the previously used protocol: 300 mV single, PP with 20 ms ISI, and varied frequencies of 1, 5, 10, 20, 50, and 100 Hz for 1 s. Stimulation sessions revealed that a variety of electric stimuli elicited particular tonic and burst responses in DCC and that in most cases they displayed preferred responsiveness to low frequency and specifically, to PP stimuli, when other forms of electric stimuli were neglected or decreased level of activity. However, in unusual conditions, greater susceptibility to other types of stimuli was also observed. In most cases, a rise in activity did not occur immediately, and some training sessions were required before the network was engaged. Unexpectedly, network activity started before occurrence of evoked responses, which only became prevalent after training sessions (Fig. 2, A, B). As a result, trained networks still showed different probability of the evoked responses that reached approximately 60 % of cases for PP stimuli ($P<0.001$, n=14), 35-40 % for single or 5 Hz stimuli ($P<0.01$, n=12 and 14), about 15-20 % for 10 Hz stimuli, up to 10 % for 20 Hz and as low as 5 % for 50 and 100 Hz stimuli ($P<0.05$, n=12, 12, 10, 9 correspondingly). Also, significant difference was found in between the groups analyses, showng a signifficantly higher effect with PP stimuli compared to all others ($P<0.05$, 0.05, 0.01, 0.01, 0.001, 0.001; correspondingly for single, 5 Hz, 10 Hz, 20 Hz, 50 Hz and 100 Hz stimuli).

During the PP stimulations, which was the most effective paradigm to elicite responses, the probability of evoked responses was around 42 % at the training phase, from which as few as 20 % of subsequent activation of the network followed. As a result, the comparison of pre-stimulus vs post-stimulus frequencies rarely reached significant difference in particular cases, when general comparison of 12 recordings showed significant difference ($P<0.001$). However, probability of evoked responses reached about 60 % during the testing phase, from which 85 % showed subsequent increase of activity level showing significant difference ($P<0.01$) at individual recordings and showng absolute effect in general comparisons ($P<0.0001$, n=12). At the same time, around 20 % did not change activity level. significant difference was found in comparisons between the training and testing phases ($P<0.001$, in single recordings; $P<0.0001$, in summarized comparisons) (Fig. 2, E, F).

We noted that when an electric stimulus coincided with enhanced network activity, especially bursts, inhibition was typically followed (Fig. 2, C, D). Around 30% of all cases PP stimuli showed inhibition of the bursting activity with significan effect ($P<0.05$, for individual cases; $P<0.001$ for n=10), when it was around 28% during the training phase, and around 25 % during the testing phase (Both with $P<0.05$). We subjected these data to the separate processing and calculations.

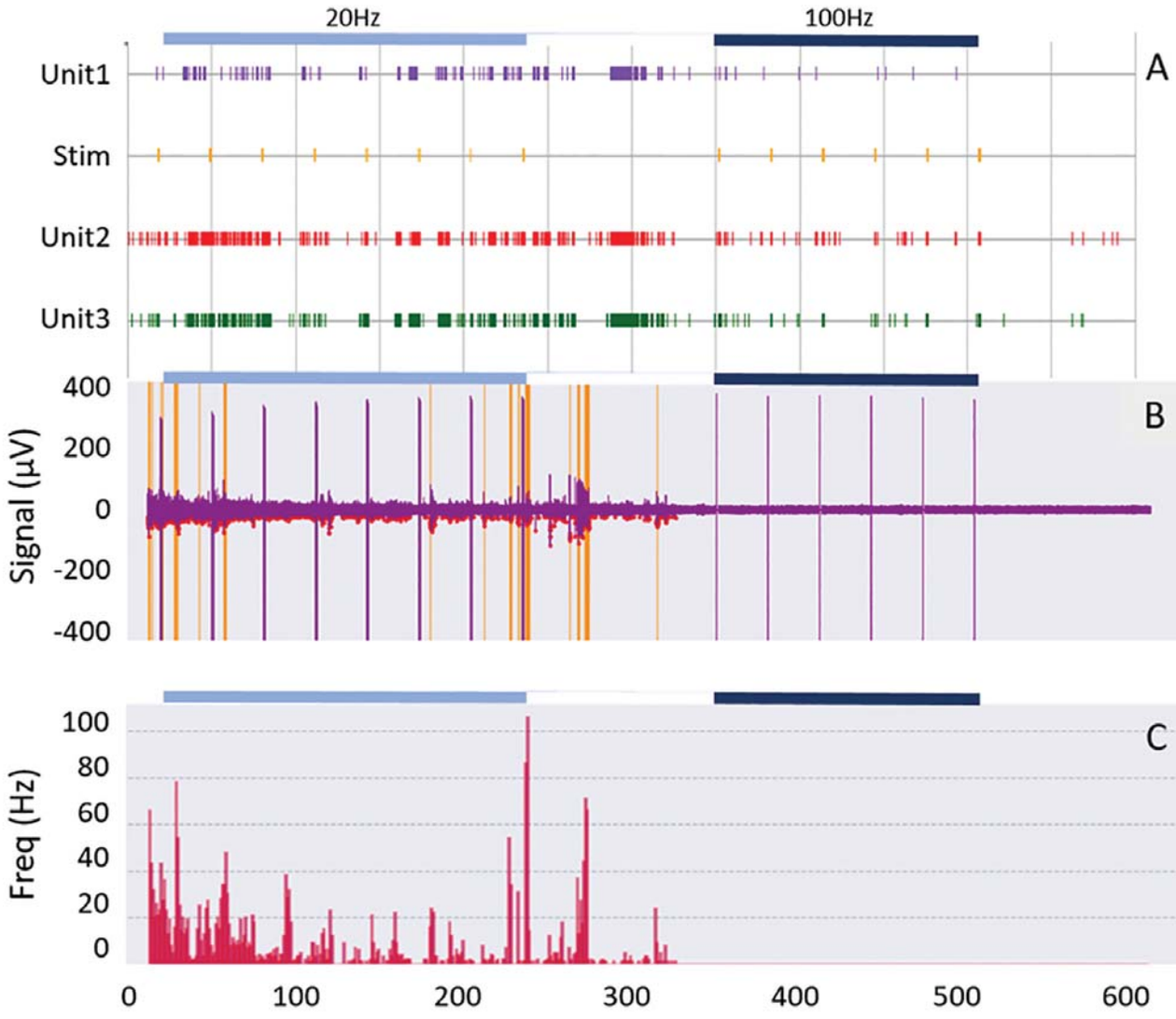

Fig. 3. *A – Example of discriminated responses to two types of applied stimuli: At the start of training, stimulation at 20 Hz (shown by the light blue line) resulted in an increase in bursting activity. Later responses reveal the creation of shorter bursts elicited by the stimuli. The recorded network is completely inhibited when 100 Hz stimuli are used (demonstrated by the dark blue line). A – Three independent single-neuron units isolated from multiunit responses (yellow spike raster indicate applied stimuli). B – Multi-unit activity of a single channel with the stimuli artifacts. The orange lines indicate the incidence of bursts. The NeuroSpace software uses red corpuscles to pick spikes for further analysis. C. Frequency bars of 0.1 s bins demonstrate absolute difference in responses to stimuli.*

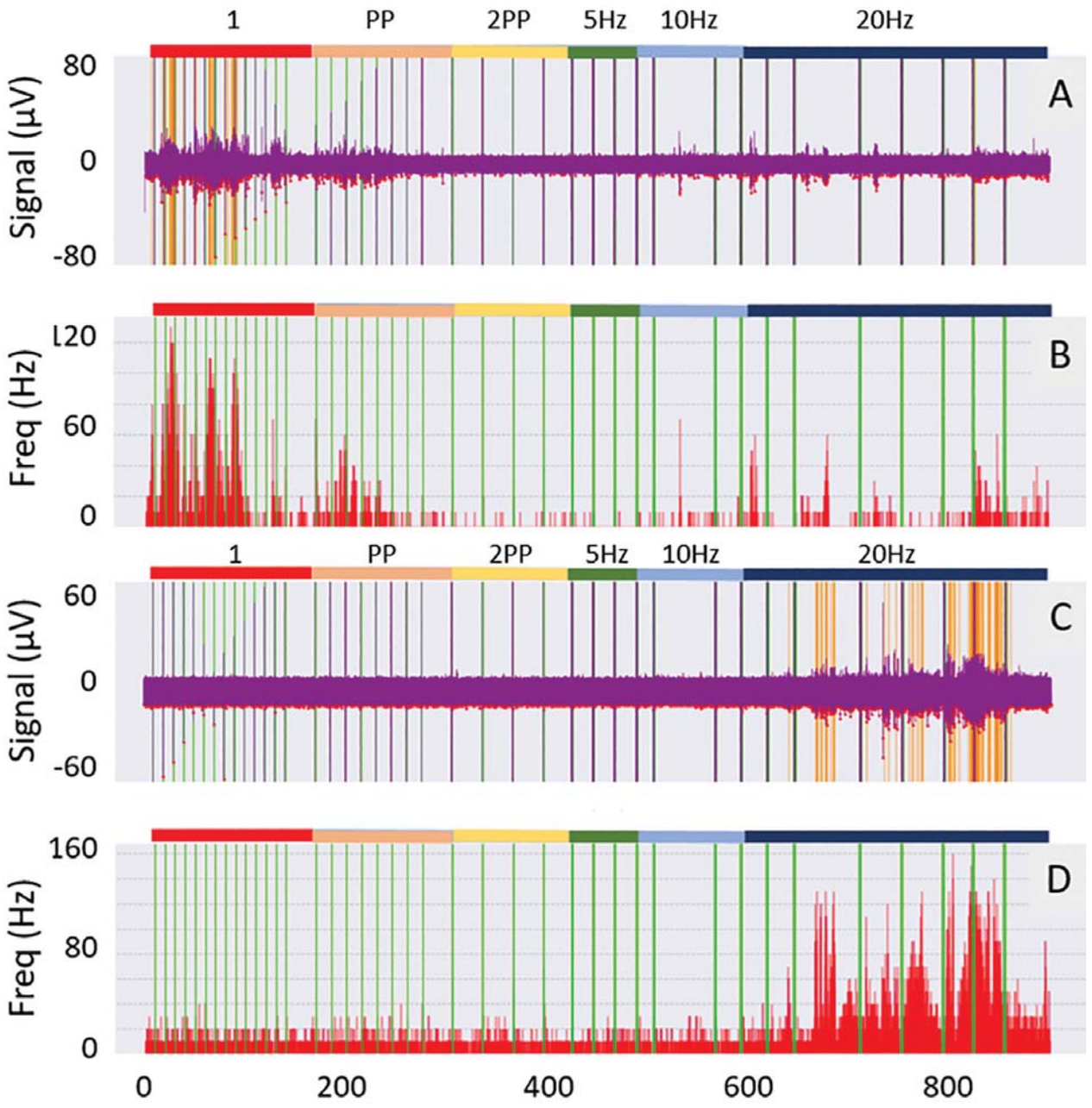

Fig. 4. *Example of mutually opposing activity in different channels during the course of an 800-second recording session, highlighting the individuality of distinct networks within a culture. The duration of each of the utilized stimuli types is indicated by colored lines above the figures: red – single stimuli, orange – paired pulses, yellow – doubled paired pulses, green – 5 Hz, blue – 10 Hz, and dark blue – 20 Hz. A, B – The channel responded to single stimuli with robust activity and continued responding to PP stimuli. The activity level was completely inhibited by double PP stimuli, which could not be restored with 5 Hz stimuli. The 10 Hz stimulation elicited some activity, which was maintained and enhanced to some extent during the 20 Hz stimulation. C, D — Another channel was completely silent throughout all other types of stimuli and was only activated when 20 Hz stimuli were delivered. Orange vertical lines on electrophysiological recording traces indicate burst activity.*

Data showed that in spite of the preference to the low frequency or PP stimuli, it was crucial question of which of stimuli were used for training of DCC. Example (Fig. 3 A, B, C) shown here demonstrates excitability of the network to the 20 Hz stimuli that was resistent, however, was absolutely inhibited by 100 Hz of stimulation. The data demonstrated that different cultures have different preferences for certain stimuli. In some circumstances, stimuli at 50 and/or 100 Hz were found to be useful in eliciting robust activity in neural networks.

In several cases, neuronal circuits within the same culture responded to the same stimuli in different ways. Arouse can be influenced by modifying the type of stimuli or the position of the stimulator electrode pairs. During the same recording session, the presented example shows reciprocally opposing activity in different channels, illustrating the uniqueness of different networks within the same DCC (Fig. 4, A, B). One of those channels responded to single stimuli with robust activity and continued responding to PP stimuli. The activity was completely depressed by double PP stimuli, which could not be restored by 5 Hz stimuli. The 10 Hz stimuli elicited some increase of activity, which enhanced to some extent during the 20 Hz stimulations. At the same time, another channel was completely silent throughout all other types of stimuli and was only activated when 20 Hz stimuli were delivered (Fig. 4, C, D).

Delayed responses to certain stimuli with a latency of more than 300 ms were also the subject of interest. The data showed that they were largely tied to the neuronal plasticity to the specific favored stimuli and had little to do with other stimuli or random cases of spontaneous activity.

Discussion

DCC's neural networks are referred to as in vivo-like in vitro system for its resamblance to the naturally developed neural circuits. MEA and related multichannel system setup make it possible to interface with DCC's neural networks in a near to the natural way, to stimulate any collection of electrodes, to record responses from the entire surface, and to analyze according long-term morpho-functional adjustments. This is an ideal environment for researching sensory perception processes at the neural network level and evaluating its information processing capabilities. In order to simulate different sensory inputs, we used a variety of electric stimuli from the same and different pairs of electrodes, recorded responses, and analyzed the

potential of these responses for coding and decoding of sensory information.

The study's main finding is that DCC's tiny neural networks are capable of retaining knowledge from discriminating sensory stimuli that served as a training element, allowing them to respond more efficiently the following time the same stimuli are presented. Typically, that function is linked to high nervous system capabilities in terms of how memory affects sensory processing and perception. This is certainly linked to cognitive functions in humans and higher vertebrates, as well as to psychological processes. Simple DCC neural networks, on the other hand, offer a large potential for achieving essentially the same functional mechanisms, where stored information can impact the acquisition of the same inputs. This also demonstrates that despite the well-known fact that the entire brain is often engaged in the realization of cognitive processes, some local circuits may provide a rather complicated support for those mechanisms.

This research relates to our recent findings that DCC neural networks can discriminate between a variety of electric stimuli that mimic sensory inputs to the recorded neural networks and respond very specifically. Sensory processing in DCC neural networks reveals a number of unique implications that are related to the trained neural networks and, secondarily, determine their impact on sensory stimuli acquisition. The consequences, on the other hand, begin with the morphological and physical nature of the neural networks in DCC, which undoubtedly determines preference for various types of stimuli [11, 12, 13]. We suggest that there should be an interplay of two related phenomena: The physical structure of certain neural circuits provides selective preference to the specified electric patterns, therefore various patterns of persistent electric stimuli can improve effectiveness to that stimuli. This logic is nicely supported by our data, which showed start of training and activated networks by tipically less effective stimuli that was inhibited by the switch to tipically favoured stimuli. That underlines the importance of the training of neural networks and previous angagement (Fig. 4 A, B).

Despite a clear preference for low frequency and PP stimuli in our research, several versions of our findings indicate that neural networks of individual DCCs or even within the same culture have the specific structural and functional basics that determine preference to certain types of sensory stimuli. Surely, specific selectivity to various electric stimuli can reveal information about the given neural network's individual features. Despite the current trend in cortical culture research to regard them as a homogeneous structures [3, 10, 12], the results of our study reveal their more complex and brain-like features, in our opinion. The reason for this could be due to the less synchronized network in our case, which was observed in general activity across the entire DCC surface.

In independent studies, DCCs have been demonstrated to be responsive to some generated low frequency stimuli and blocked by high frequency stimuli [11, 12]. High frequency bursts, such as 20 Hz, were employed in certain studies to induce neuronal

plasticity in DCC networks [13]. The most plasticity changes in our experimental setup were caused by PP activation. In our case, 20 Hz training did not yield the best results for increasing plasticity, whereas PP stimuli did. There is no disagreement in our perspective, however it depends on the specificity of the neural network employed in the study. We observed that divergence in the nature of DCCs in our circumstances.

Furthermore, our findings support Nieus and colleagues' recent findings in hippocampal neural cultures [14] regarding state-dependent representation of stimulus-evoked activity. In our recordings, it was frequently noted that when stimulus occurrence corresponded with elevated activity levels, particularly bursts, subsequent evoked responses were blocked. These cases certainly needed to be looked at independently from the cases of evoked responses in order to make sense of them and avoid losing their meaning. From a medical aspect, it could play a significant role in seizure disorder care in the near future.

Many of our recordings revealed progressive activation of neural networks, indicating gradual degrees of neural circuit involvement in information processing. Training aids the formation of synapses [1], which is an important part of the proper development of brain tissue. Simultaneously, as established in our study, it enhances the likelihood of evoked responses in the presence of favorable stimuli.

Instant and delayed evoked responses should reflect immediate and delayed information processing, with the likelihood of rapid responses increasing at training sessions with preferred stimuli. It shows how synaptic plasticity affects memory formation in response to positive sensory input. At the same time, it emphasizes how information is processed in a sequential manner in order to be memorized. Our findings suggest that subsequent responses are also important for stimulus discrimination, which could be a reflection of the long-term neuroplasticity processes that could aid information coding, particularly in developing neural circuits. The generation of later responses is likewise dependent on stimulus specificity, and they are not existent before they are administered, according to the data. There are few findings in the literature for those reactions, with the focus being on evoked responses with a short latency. However, few studies have highlighted the importance of delayed reactions in brain plasticity processes [15].

The data shows how small neural networks process information in response to a wide range of sensory events. It reveals that they have the ability to select stimulus patterns that are appropriate for their physical nature and, if repetitive stimuli are presented, to process brain plasticity changes that, in turn, influence asquisition of the same stimuli more effectively. This demonstrates that small neural networks play an important part in the nervous system's high-level processes.

Conclusions

- The neural networks of DCC are capable to memorize discriminated sensory stimuli;
- Memorized information in the neural networks of DCC impacts acquisition of the favored stimuli;
- Small neural networks may be able to help high nervous functions solve a major portion of complex tasks.

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