WHAT CAN WE LEARN BY CONNECTING NEURONS IN A NANODEVICE?

A short overview and some proposals

Alfredo Colosimo
Sapienza, University of Rome, CISB Research Center
colosimo@caspur.it

Claudio Frank
Istituto Superiore di Sanita' (Rome)
claudio.frank@iss.it

Keywords: Nanodevices; Neuron Growing, Cocultures.

Abstract: A brief overview of examples selected from the literature and concerning structural and functional properties of neurons explored by systematic use of nanodevices is provided, in the aim to show that: i) the native clustering ability of isolated neurons can be controlled and induced to form clusters of interconnected functional units on appropriately engineered nanodevices; ii) the initiation of Synchronized Bursting Events (SBE) by local chemical stimulation can be evidentiated in cultured neurons using Micro Electrode Arrays (MEA); iii) the interactions among different cell types (cocultures) can be studied in compartimentalized microfluidic platforms where different microenvironments are separated and let communicate through microchannels. Over and above their intrinsic value, such exemplary cases inspired our own research projects on cocultures of electrically excitable cells. Our main target are the mechanisms of axon-localized neuronal damage, as arising in some rare neurodegenerative pathologies as well as in Amiothrophic Lateral Sclerosis (ALS). In this frame, clarifying the sequence of events initiating the formation of structural interactions between neurons and miocytes is mandatory, and even mimicking the Neuromuscolar junctions appears feasible.

1 INTRODUCTION

It could appear hazardous to state that the logical link between cloud (/bubble) chambers and particle accelerators is pretty similar to that between electron (/atomic force) microscopes and nanofluidic devices. At a closer view, however, one should admit that both cloud chambers and ultramicroscopes increased in unprecedented way the resolution power in visualizing physical and biological entities, respectively. On the other hand, accelerators and nanofluidic devices gave us the opportunity to control and modify their behaviour, at a comparable dimensional scale, particularly as far as the reciprocal interactions are concerned. The issue is of great theoretical and practical impact in any scientific discipline, since it makes easier the transition from purely descriptive to predictive models at a more fundamental level.

Within Biology, this is even more so in dealing with neurons, a type of cells whose characteristic features stem from the complex network of chemical and physical interactions among individual cells and their neighbours, of the same or of different type. Nowadays it is commonly accepted that neurons realize in the human Central Nervous System (CNS) probably the most sophisticated and complex object in the known universe. At the same time, any relevant neurons' misfunction, either of psychiatric (e.g. schizofrenia) or neurological (e.g. ALS) nature, reveals extremely difficult to recover. The two facts are obviously linked, since the complexity level is by definition inversely related to the efficacy of our predictive models and control abilities. Does it mean that any human effort in this direction is hopeless? May be not, if we appropriately down scale (i.e. simplify) the phenomena of interest. Only upon reduction to the minimum possible number the essential components of the experimental set-up, our scientific endeavour may be pursued with reasonable confidence. Such considerations naturally open the door to a systematic use of nanodevices in which microfluidic can insure continuous dynamic control of the experimental conditions.

In this contribution we report about a research program designed around the still unknown ethiopathology of quite rare and/or severe neurological pathologies like ALS. By means of appropriately engineered microfluidic devices, we can separate into different compartments the neuronal cells from other cell populations (e.g. glia, myocytes, etc.): this allows a precise control of the traffick of chemical and electrical messages across different cell types and of the noxious or beneficial effects of various drugs.

The problems we decided to tackle and the related experimental strategy will be introduced after a short preliminary discussion of a few exemplary cases taken from the recent literature and relevant to our context.

2 EXEMPLARY CASES

2.1 Formation of neuron clusters and axonal connections on a nanodevice.

The tendency to aggregate is a well known feature of neurons under *in vitro* growing conditions, and surface texture at the scale of tens of nanometers to micrometers can influence the attachment of cells to quartz or glass substrates. An obvious indicator of the good conditions of neurons is the ability to aggregate and to extend axonal connections among each other, and a number of precautions to facilitate the adaptation and survival of neurons over a relatively long time span (in the range of weeks) in the exotic environment constituted by a nanodevice, are reported in the literature (Yeon and Park, 2007). Since 2005 Gabay and collaborators (Gabay et al., 2005) demonstrated that the formation of neuron clusters can be directed towards tiny wells carved on a quartz nanosurface according to a predefined geometry (Figure 1). The necessary chemical pretreatment of the device involves coating the wells by a fine texture of carbon nanotubes (CNT), which probably makes them attractive for cellular membranes due to hydrophobic forces. From each cluster, the gangliated neurons send neurites towards nearby clusters, thus forming interconnected networks whose electrical viability can be checked by standard patch clamp techniques (Patolsky et al., 2006).

2.2 Arising of synchronized electrical activity

The fine functional features of neural cultures can be inspected by means of Micro Electrode Arrays (MEA) (Boven et al., 2006). Electrodes are composed of indium tin oxide or titanium, with diameters between 10 and 30 μm. Spatial resolution is one of the key advantages of MEAs and allows signals sent over a long distance to be taken with higher precision when a high-density MEA is used. MEAs usually have a square grid pattern of 256

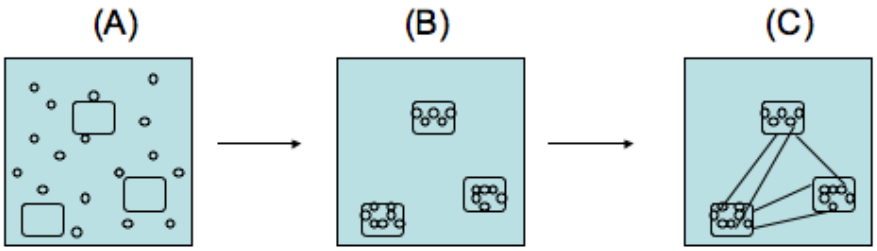

Figure 1: *Aggregation of neurons in a predefined lattice of CNT coated islands.* According to Gabay et al. (2005), regular arrays of hydrophobic carbon nanotube islands (in the order of 100 μm) can be grown on hydrophilic SiO_2 or quartz substrates to form preferred surfaces for neuronal adhesion. Neuronal clusters are formed as the cells migrate on the low affiinity substrate (quartz) towards high affinity, lithographically defined carbon nanotube templates on which they adhere and assemble.(A) Initial random distribution of neurons as seminated on a patterned quartz surface; (B) spontaneous clustering into CNT coated islands; (C) Formation of axonal connections among islands. In the original paper (Gabay et al., 2005) the distance between the islands is around 150 μm, the average neurons/island ratio is 40, with an initial cell density of $1 * 10^3/mm^2$, and the time span from (A) to (B) and from (B) to (C) is of the order of 4 days and 3 days, respectively.

electrodes that cover an area of 2.8 by 2.8 mm. Using a specific type of MEA with (6 x 10) electrodes at a distance of about 200 μm from each other Baruchi and Ben-Jacob in 2007 observed quite peculiar patterns in the electrical response following repeated chemical stimulation of areas of size comparable to that of the electrodes grid. The recorded signals clearly show that repeated stimulations of the same area generates a practically synchronous electrical activity involving a consistent number (several dozens) of neurons along a well defined spatial pathway. Moreover, such ordered and synchronous spatial patterns persist almost unaltered for several hours and are clearly distinct from the background electrical activity randomly distributed in space and time and always present within any vital neural population.

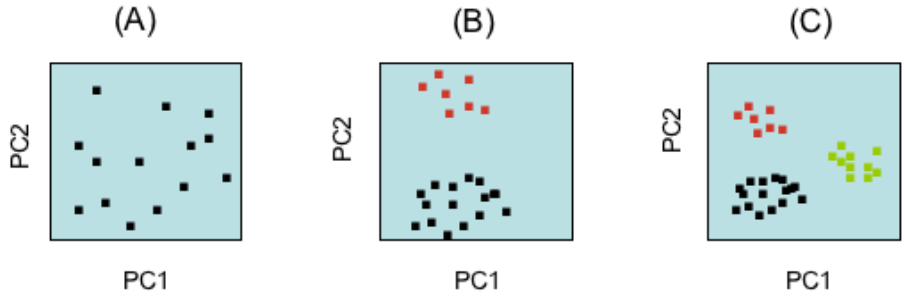

Figure 2: *Electrical activity of cultivated neurons before and after repeated chemical stimulation.* The highly schematized drawing (from a figure of Baruchi and Ben-Jacob, 2007) visualizes, in the space of the first and second principal components, the classes of SBE generated in the absence and in the presence of appropriate chemical stimulations (10 μl droplets of 100 μM picrotoxin), and separated by a dendrogram hierarchical clustering method. Such clustering method was applied to the cross correlation values of any couple of activity vectors recorded by the 60 microelectrode MEA used by the authors. (A) Before the chemical stimulation: the signals (black points) refer to the background electrical activity. (B) After the first train of 20 repeated stimulations at $20''$ intervals, a well defined cluster of synchronized signals (red points) appears. (C) After 24 hrs a second (identical but in a different location) train of stimulations was applied, and a second cluster of symbols (green points) appears, clearly distinct from the (always present) background and from the persistent previous one.

Figure 2 (from Baruchi and Ben-Jacob, 2007, Fig. 3, modified) shows the result of a principal component analysis of the observed activity patterns and indicates that the response to a given train of stimulations in the space of the first two chemical components does not substitute but actually coexists with the response to a previous train. This provides a consistent and reliable, although necessarily oversimplified, mechanistic model of medium- and long-term memory representations.

May be the most impressive conclusion from the cited work (Baruchi and Ben-Jacob, 2007) deals with the realistic simulation and interpretation of complex physiological functions which can be realized by analyzing high spatial resolution data in the electrical records from multiple electrodes, through appropriate multivariate statistical procedures.

2.3 Cocultures studied by nanodevices.

Shifting the target of nanotech applications from relatively homogeneous cell populations to different cell types, implies a substantial complication in the underlying cell manipulation techniques, as well as a peculiar architecture of nanodevices ad hoc designed for a given experiment, not to mention the need for a precise control of the minute amounts of fluids surrounding each cell type. As a matter of fact, recent improvements in polymer technologies for manifacturing microfluidic devices (Abgrall and Gue', 2007) appear very promising in order to meet the requirement of specific chemical environments for the survival of each cell type, coupled to a fine control of the traffic of chemical messages among similar or different cells.

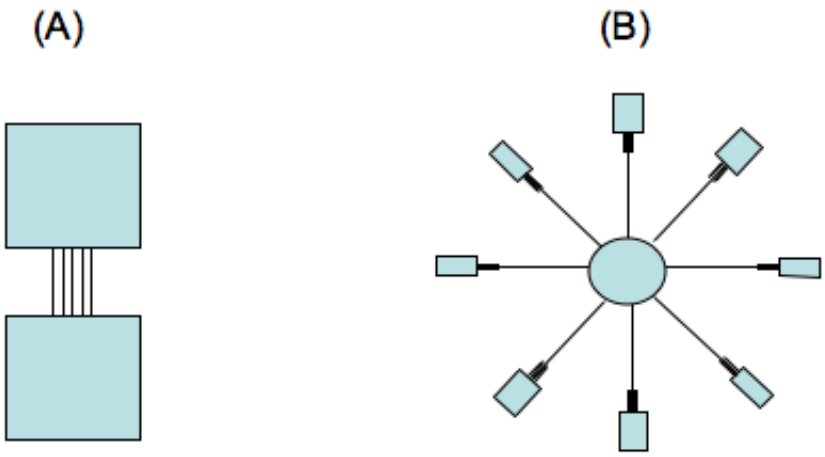

Figure 3: *Microfluidic platforms for cell cocultures.* (A): The upper and lower chambers may host different cell types (for example, neurons and glia): the tiny channels connecting them can be made pervious to axons only, or to monodirectional flux of fluids. (B): The cell population in the central chamber can be connected to one or more surrounding chambers through the corresponding channel(s): The channels have similar features and functions than those in (A).

Figure 3 contains an oversimplified schematics of the minimum architectural features of a multichamber nanodevice. Similar architecture have been used to study axons-glia interactions (Hosmane, 2010). In that paper it is shown that that glia's motility is enhanced towards axons pertaining to necrotic or injured cell bodies. The implications in modeling the maintainance and progression of a number of neuroinflammatory or neurodegenerative deseases are straightforward.

3 OBSERVING INTERACTIONS AMONG EXCITABLE CELLS IN A NANODEVICE.

Besides the ability to trace the event of interest down to the level of single cells, the advantage of using nanotech in studying the interactions among excitable cells is at least twofold: first, we can assign to each cell population a defined location where to settle and operate; second, the transfer of chemical or physical messages among the various cell locations can be precisely tuned in both size and direction.

The possibility to isolate from the chemical point of view two different areas by means of a light flow, and to allow synaptic connection between the neurons in the different areas, is a relevant added value of this approach. In other words, the distinction between pre- and post-synaptic events becomes much easier than in a bulk mixture of cells, since they can occur in different compartments only connected by axons, and among which the transfer of chemicals can be made unidirectional by microfluidic control (see below).

3.1 Synaptic transmission studies.

Impairment of synaptic transmission, due to disorders in neurotransmitters receptors activity, has a primary role in many neurological diseases. A reduced sensitivity of these receptors, e.g. to excitatory glutamatergic agonists, can decrease the efficacy of central nervous system (CNS) and reduce or block any motor performances of patients. The study of excitatory receptors can be useful in a wide spectrum of neurological diseases, including rare diseases with clinical symptoms such as loss of memory and learning, or motor deficits.

A very useful tool to study the activity of excitatory aminoacid receptors in cultured neuronal cells is given by the measure of intracellular calcium concentration $[Ca^{2+}]$ using fluorescent dyes. In fact, when glutamatergic agonists are applied to neurons, a fast increase of $[Ca^{2+}]$ is induced. These experiments allow to evaluate the functional activity of the receptors, and the influence of drugs able to interact with them. This is generally cosidered as a "postsynaptic" model: the application of drugs to all the cells at the same time simulates what happens after synaptic release.

A limit of these studies on cultured cells is that neurons connected to each other by synaptic connections are in the same compartment, and if a drug is applied in the medium, chemical stimulation reaches all of them at the same time. This impairs detecting possible alterations in the synaptic communications between individual cells. For example, if the transmission of signals between two cells is impaired due to a reduced pre-synaptic neurotransmitter release, this will be undetectable by studies on intracellular calcium concentration, since both pre- and post-synaptic cells will be stimulated by the external agonist at the same time.

Such a limit can be overcome by a nanodevice. The possibility to divide the space where the cultured cells live (e.g. a Petri dish) into distinct compartments, connected by tiny channels only pervious to axons, allows to apply chemical substances to one compartment, and exclude its presence in the other compartments. Under these conditions, if we stimulate a neuron (1) by an excitatory amino acid, we induce the activation of an action potential by a post-synaptic mechanism. If we now focus on neuron (2) lying in a chemically distinct compartment and still in synaptic connection with neuron (1), any change induced on neuron (2) by the excitation of neuron (1) will be due to synaptic transmission. As a consequence, this approach allows to single out, in a synaptic transmission, both pre- and post-synaptic events.

3.2 Interactions among neurons and myocytes.

3.2.1 Generalities

Due to the incomplete information available in the field, any improvement in understanding the complex interaction mechanisms of neurons with other cells (glia, myocytes, leucocytes, etc) is most welcome. In addition, the topic looks quite a favourable case to explore by means of microfluidic devices, since they easily allow handling cocultures of different cell types in different compartments at the nanoscale. Finally, it is difficult to overestimate the practical relevance of any new information on the cellular and molecular events underlying the neurons' functions for the design of appropriate strategies against the many pathological conditions in which they are compromised or injured.

3.2.2 ALS: background

In particular, we deserved special attention to Amyotrophic Lateral Sclerosis (ALS), sometimes called Lou Gehrig's disease. ALS is a rapidly progressive, invariably fatal disease that attacks the nerve cells responsible for controlling voluntary muscles. The disease belongs to a group of disorders known as motor neuron diseases, which are characterized by the gradual degeneration and death of motor neurons. There is a known hereditary factor in Familial ALS (FALS): an inherited genetic defect on chromosome 21 (coding for superoxide dismutase, SOD1), is associated with approximately 10% of familial cases of ALS. however, there is no known

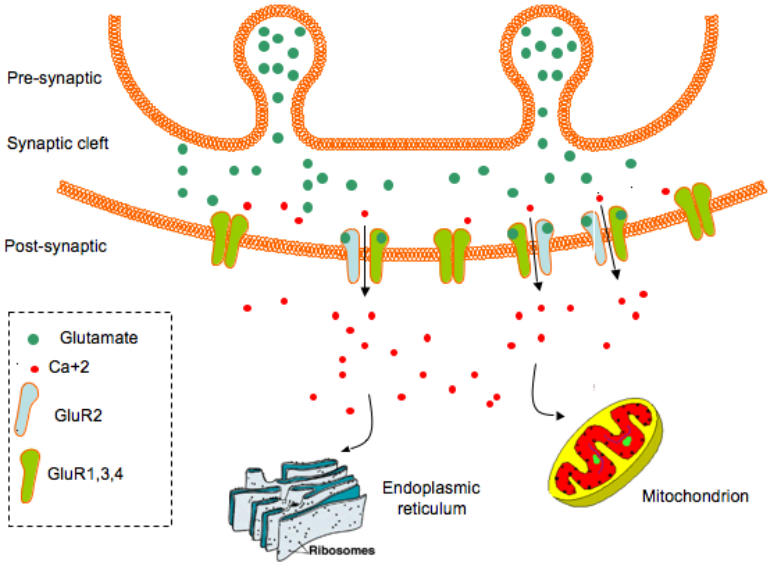

Figure 4: *Calcium dysregulation in motor neurons.* Ca^{2+} enters the post-synaptic neuron through the mutant AMPA glutamate receptor in which the mutated subunit (GluR2) has greatly enhanced Ca^{2+} permebility. The noxious effects of Ca^{2+} are probably exerted at the level of mitochondria and of the endoplasmic reticulum, where it tends to accumulate (Grosskreutz et al., 2010).

hereditary component in the $90-95\%$ cases diagnosed as sporadic ALS (SALS). For the latter group a variety of possible causes have been proposed in the last decades, including, since the late 1980s, the impaired production, release, and/or uptake of neurotrophic factors (Ekestern, 2004), and, more recently, the multifaceted origins and consequences of oxidative damage (Barber and Shaw, 2010). As for neurotrophic agents, however, none of the encouraging results of the *in vitro* tests, was confirmed by extensive clinical trials. Concerning the antioxidants, essentially the same conclusion is reached by Barber and Shaw (2010) who recognize that, although some antioxidants have shown beneficial effects in animal models, human clinical trials of antioxidant therapies have so far been disappointing. The same authors underline the emerging picture of many complex, multifactorial, and incompletely understood pathogenic processes contributing to the arisal and progression of ALS. Thus, what is clear is that motor neuron death occurs not as the result of a single insult, but rather through a combination of mechanisms including not only oxidative stress, but also excitotoxicity, mitochondrial dysfunction, endoplasmic reticulum stress, protein aggregation, cytoskeletal dysfunction, involvement of nonneuronal cells, and defects in RNA processing and trafficking.

In such an intricate and intimidating landscape, a unifying concept has been recently suggested by Grosskreutz (Grosskreutz et al., 2010) who indicates the central role of calcium dysregulation in the pathophysiology of ALS. This proposal has been stimulated by the following observations: 1) one of the most striking characteristics of ALS is the selectivity of the disease process for motor neurons; 2) excitatory neurotransmitters, in particular glutamate, can induce neuronal death because motor neurons are extremely susceptible to excitotoxicity. 3) Spinal motor neurons receive very strong glutamatergic input, express Ca^{2+} permeable (AMPA) [1] receptors on their surface and have a low Ca^{2+} buffering capacity.

The latter characteristics are most likely crucial for motor neurons to be functionally normal, but under pathological conditions they could cause or speed up motor neuron death. In particular, it as been noted that the Ca^{2+} permeability of AMPA receptors is determined by the absence or presence of the GluR2 subunit in the receptor complex. Under most conditions, AMPA receptor tetramers contain at least one GluR2 subunit and have a very low Ca^{2+} permeability, while GluR2-lacking receptors are highly permeable to Ca^{2+} (Figure 4).

An even more interesting and intriguing proposal (Das et al., 2007) points to the NMJ malformation/misfunction as the key event in ALS, on the basis of the best currently available ALS animal model for

[1] -amino-3-hydroxy-5-methylisoxazole propionic acid

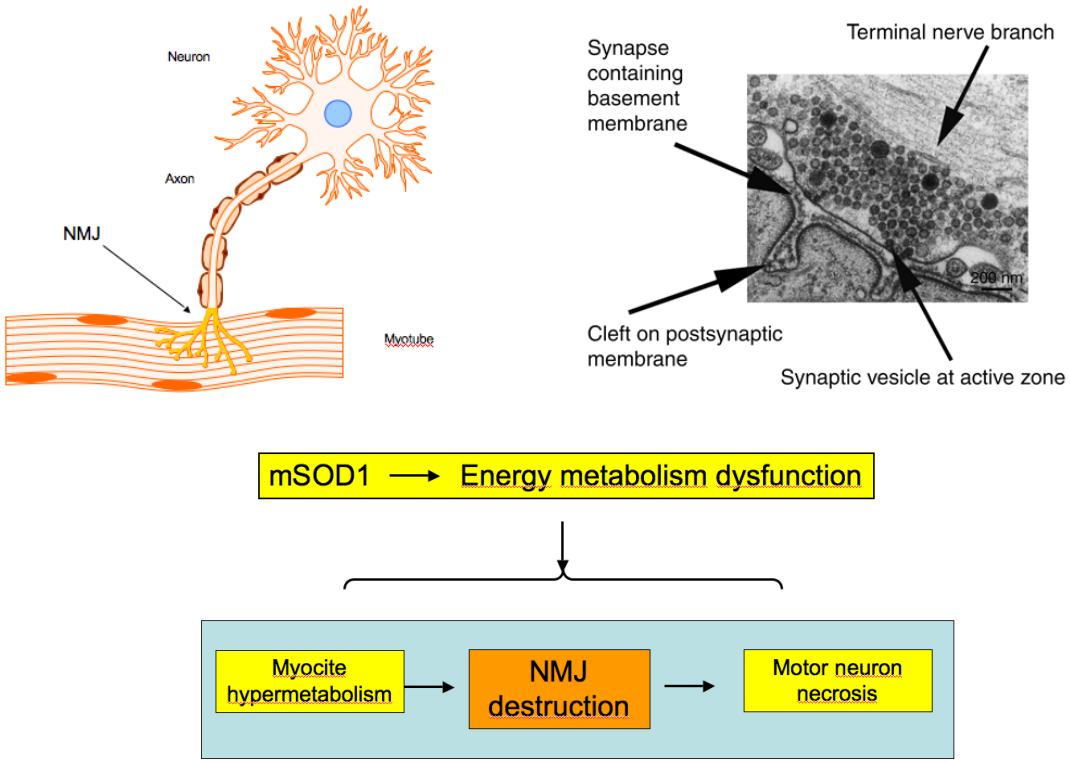

Figure 5: *NeuroMuscolar Junction* The neuromuscular junction (NMJ) is a structure in charge of a rapid and efficient connection between neuron and myotube. This is achieved by the close spatial apposition of the presynaptic zone, containing synaptic vesicles filled with the neurotransmitter acetylcholine, to the postsynaptic muscolar membrane. UPPER LEFT: A highly schematized NMJ. the modulatory role played by a third cell type, the Schwann cells is not shown. UPPER RIGHT: Highly magnified view of a neuromuscular junction from: (Hirsch, 2007). BOTTOM: Hypothetical mechanism of NMJ destruction as a consequence of an mSDO1 induced metabolic disfunction acting primarily on myotubes.

preclinical trials (Dupuis and Loeffler, 2009): mice overexpressing the mutant form of the SOD enzyme (mSOD1 mice). The first process in the disease of these mice is the destruction of the NMJ, followed by degeneration of the axon and, finally, of the motor neuron cell body by apoptosis. However, even a complete rescue of neuron cell bodies from apoptosis could only marginally affect muscle denervation and delay animal death (Gould et al., 2006). From these results it has been concluded that the relevant pathogenic effect is not the neuron death by itself, but rather the loss of motor-neuron contacts (Dupuis and Loeffler, 2009). As for the cause of NMJ destruction, the same authors suggest that: 1) abnormalities in muscle energy metabolism could be the direct cause, since in the mSOD1 mice model a chronic energy deficit has been observed, which precedes amyotrophy and muscle denervation, and 2) the energy metabolism dysfunction could concern not only the motor units, but also cause a number of systemic alterations (lipid metabolism, hepatic gluconeogenesis, ec.) (Figure 5, bottom panel).

In spite of their great importance, conclusions from animal models do not entitle straightforward extension to human diseases, and their ultimate validation obviously relies upon an appropriate set of expensive and time consuming clinical trials. An intermediate step, however, extremely useful in pruning and/or refining mechanistic hypotheses and therapeutical strategies, remains the *in vitro* reproduction of events which are supposed relevant in a given theoretical/modelistic context. In the specific case of interest, namely the formation/destruction of the NMJ, a substantial improvement in the quality and quantity of the available information is expected from studies of the neuron-myocyte interaction at the single cell level and under accurately controlled conditions, by systematic use of appropriate microfluidic nanodevices (see below).

3.2.3 ALS: a basic research program

Given the complexity of the neurodegenerative processes involved in ALS and the extremely difficult dissecting cause and effect, any research program in the field should very clearly indicate the working hypotheses underlying the planned experimental protocols. In our case, we decided to use appropriately designed nanodevices of the type sketched in Figure 3 and to concentrate on the following specific points:

A) formation of NMJ-like structure in microscale cocultures of rat neurons and human myotubes, separately located in their own wells and interacting by axonal connections through appropriate microchannels. This is the preliminary and most important step, in which neurons and myocytes will be induced to form stable aggregates in separate round chambers of approximately 200 μm diameter, engineered on a PDMSO plate according to the method described in (Gabay et al., 2005). In both cases a minimalist serum-free medium will be used, optimized for co-culture of rat mammalian nerve and muscle cells in order to form functional neuromuscular junctions, following (Das et al., 2007). The most appropriate cell density for developing axonal connections will be chosen in the range of $(1-10)*10^3/mm^2$. In a time window of 2 -5 days, the axons protruding from the neuron chamber are expected to reach the myocytes in the other chamber, through the intermediate microchannels and establish functional connection with them.

B) Studying the role of factors conditioning a functional innervation. In macroscale cocultures, such factors span from Schwann cells (Guettier-Sigrist et al., 2000) to NGF (facilitating factors) and include alfa-tubocurarine and excitoxic agents (destructive factors). The added value of a coculture on a microfluidic device is related to i) the non-ambiguous discrimination between pre- and post-synaptic effects, and ii) to the high spatial and temporal resolution of the observed patterns. Such opportunities, for example, will be precious to test the hypothesis (Fig 5, bottom panel) that the events causing the destruction of the NMJ are initiated in the muscular tissue and only eventually lead to the motor neuron death.

The data acquisition techniques which we will prefentially (but not exclusively) use include: fluorescence spectroscopy, to get information over intracellular Ca^{2+} levels, and electrophysiological records from appropriate MEA devices coupled to direct microscopic observations. These two equipment form the basic experimental set-up for any study of structure-function relationship in excitable tissues. This applies, in particular, if the approach is based upon investigations at the micro scale, where subcellular or membrane events are in principle accessible and under control, if the resolution power of the used equipment allows. Moreover, in the case of our concern it is immediate to realize that the synaptic development or the NMJ formation, besides the highest possible resolution in space and time, demand the synchronous acquisition of the two types of data. It would be much more difficult, if not impossible, otherwise: 1) to propose a realistic and convincing model for the dynamics of the synaptic vesicles approaching the pre-synaptic side of the Junction, and 2) to correlate the delay (or the absence) of the ensuing electric signal on the post-synaptic side, to the normal/impaired function.

4 CONCLUSIONS

For a well-balanced consideration of both advantages and possible problems linked to the use of nanodevices in studying cell-cell interactions, it is worth reminding the following viewpoints:

- On one hand, it has been claimed that: .... *microfluidic cell culture systems convey more reliable results due to their ability to grow cells as biological systems do, and because they outperform those from conventional cell cultures and assay systems.....* (Yeon and Park, 2007). Such an enthusiastic statement is essentially justified by the observation that microfluidic technology can be used to supply and transfer media, buffers, and even air while the waste products by cellular activities are drained in a way resembling the human circulatory system.

- On the other hand, a more recent review underlines that in order to integrate biological data obtained via traditional methods with results obtained in engineered microenvironments, one should pay attention to a manifold of possible artifacts hidden in a rushed exploitation of such a sophisticated technology. According to (Paguirigan and Beebe, 2009), *...even a simple microfluidic channel used for cell culture can cause changes in the cellular microenvironment influential enough to alter or mask the true cellular responses to stimuli....*

In our opinion, however, in such a complex network of problems enthusiasm as well as skepticism should be avoided. Accordingly, it would be a wise strategy to take into account both the above viewpoints by a working team including biologists and engineers. They could exploit at best the great potential of nanodevices and minimize their pitfalls through a continuous exchange of ideas and consideration of difficulties, in a fully collaborative

effort towards solutions.

ACKNOWLEDGEMENTS

Sincere thanks are due to the colleagues who directly or indirectly made this contribution possible and, in particular, to Luca Businaro and AnnaMaria Gerardino.

REFERENCES

Abgrall, P. and Gue', A. (2007). *Lab-on-chip technologies: making a microfluidic network and coupling it into a complete microsystem - A review*. J. of Micromechanics and Microengineering; 17:R15-R49.

Barber, S. and Shaw, P. (2010). *Oxidative stress in ALS: Key role in motor neuron injury and therapeutic target*. Free Radical Biology and Medicine, 48: 629641.

Baruchi, I. and Ben-Jacob, E. (2007). *Towards neuro-memory-chip: Imprinting multiple memories in cultured neural networks*. Physical Review E, 75: 050901.

Boven, K.-H., Fejtl, M., Mller, A., Nisch, W., and Stett, A. (2006). *On Micro-Electrode Array Revival. in: (Baudry M, Taketani M, eds.) Advances in Network Electrophysiology Using Multi-Electrode Arrays*. New York: Springer Press; 24-37.

Das, M., Rumsey, J., Gregory, A., Bhargava, N., Kang, J., Molnar, P., Riedel, L., Guo, X., and Hickman, J. (2007). *Embryonic motoneuron-skeletal muscle co-culture in a defined system*. Neurosci., 146:481-488.

Dupuis, L. and Loeffler, J. (2009). *Neuromuscular junction destruction during amyotrophic lateral sclerosis: insights from transgenic models*. Curr. Opin. Pharmacology, 9:341-346.

Ekestern, E. (2004). *Neurotrophic Factors and Amyotrophic Lateral Sclerosis*. Neurodegenerative Dis. 1: 88100.

Gabay, T., Jakobs, E., Ben-Jacob, E., and Hanein, Y. (2005). *Engineered self-organization of neural networks using carbon nanotube clusters*. Physica A 350: 611-621.

Gould, G., Buss, R., Vinsant, S., Prevette, D., Sun, W., Knudson, C., Milligan, C., and Oppenheim, R. (2006). *Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS*. J Neurosci, 26:8774-8786.

Grosskreutz, J., Bosch, L. V. D., and Keller, B. U. (2010). *Calcium dysregulation in amyotrophic lateral sclerosis*. Cell Calcium 47, 165174.

Guettier-Sigrist, S., Coupin, G., Warter, J.-M., and Poindron, P. (2000). *Cell Types Required to Efficiently Innervate Human Muscle Cells in Vitro*. Experimental Cell Research 259, 204212.

Hirsch, N. (2007). *Neuromuscular junction in health and disease*. British Journal of Anaesthesia 99: 132-138.

Hosmane, S. (2010). *Circular compartmentalized microfluidic platform: Study of axonglia interactions*. RSC Publishing.

Paguirigan, A. and Beebe, J. (2009). *From the cellular perspective: exploring differences in the cellular baseline in macroscale and microuidic cultures*. Integr. Biol., 1: 182-195.

Patolsky, F., Timko, B., Yu, G., Fang, Y., Greytak, A. B., Zheng, G., and Lieber, C. M. (2006). Detection, stimulation, and inhibition of neuronal signals with high-density nanowire transistor arrays. SCIENCE, 313: 1100.

Yeon, J. and Park, J. (2007). *Microfluidic Cell Culture Systems for Cellular Analysis*. Biochip Jornal, 1: 17-27.

APPENDIX 1
RECENTLY COMPLETED RESEARCH PROGRAMS CONCERNING ALS.

Many research programs have been dedicated to try and understand the mechanisms that triggers selective motor neurons degeneration in ALS and to find effective strategies to halt the processes leading to cell death. In most cases, however, the results of clinical trials were not satisfactory (see Figure 6). Such programs mainly included studies in animal models aiming to clarify why and how SOD1 mutations lead to the destruction of neurons. The excessive accumulation of free radicals implicated in a number of neurodegenerative diseases including ALS, is also being actively studied, as well as the loss of neurotrophic factors or the presence of specific

Drug	Current situation	Producer
Myotrophin®	Study Completed	Cephalon
Celebrex	Study Completed	Northeast ALS Consortium
NEOTROFIN™	Study Completed	NeoTherapeutics, Inc.
Neurodex	Study Completed	Avanir Pharmaceuticals
Rilutek®	FDA approved drug currently available	Aventis S.A.
Oxandrolone	Study Completed	The Carolinas Neuromuscular/ALS MDA Center
Pilot CoQ10	Enrollment Closed	Eleanor and Lou Gehrig ALS Center at Columbia University
High Dose Coenzyme Q10	Enrollment Closed	The Eleanor and Lou Gehrig MDA/ALS Research Center
Pilot CoQ10 with imaging	Enrollment Closed	Eleanor and Lou Gehrig ALS Center at Columbia University
Topiramate (Topamax)	Study Completed	North Eastern ALS Consortium
Xaliproden	Preliminary analysis released 9/5/00	Sanofi-Synthelabo Inc.
Indinavir	Study Completed	Beth Israel Medical Center
Creatine	Study Completed	North Eastern ALS Consortium
Minocycline	Enrollment Closed	Eleanor and Lou Gehrig ALS Center at Columbia University

Figure 6: Notice that the only currently available drug is Rilutek (Riluzol) since it somehow survived the clinical trials. It delays the onset of ventilator-dependence or tracheostomy in selected patients and may increase survival by approximately 3 to 5 months. Riluzole preferentially blocks TTX sensitive sodium channels, which are associated with damaged neurons. This reduces influx of calcium ions and indirectly prevents stimulation of glutamate receptors. The table has been compiled using information available at *http://www.alsa.org*

neurotoxic agents. Exploring the action mechanisms of these and other possible factors at the molecular and cellular level by means of microfluidic nanodevices should increase the resolution power of the observations and the similarity of the *in vivo* systems with the *ex vivo* conditions. Thus, it should provide essential contributions to clarify at least some of the causes of motor neuron degeneration in ALS and to design effective therapies.