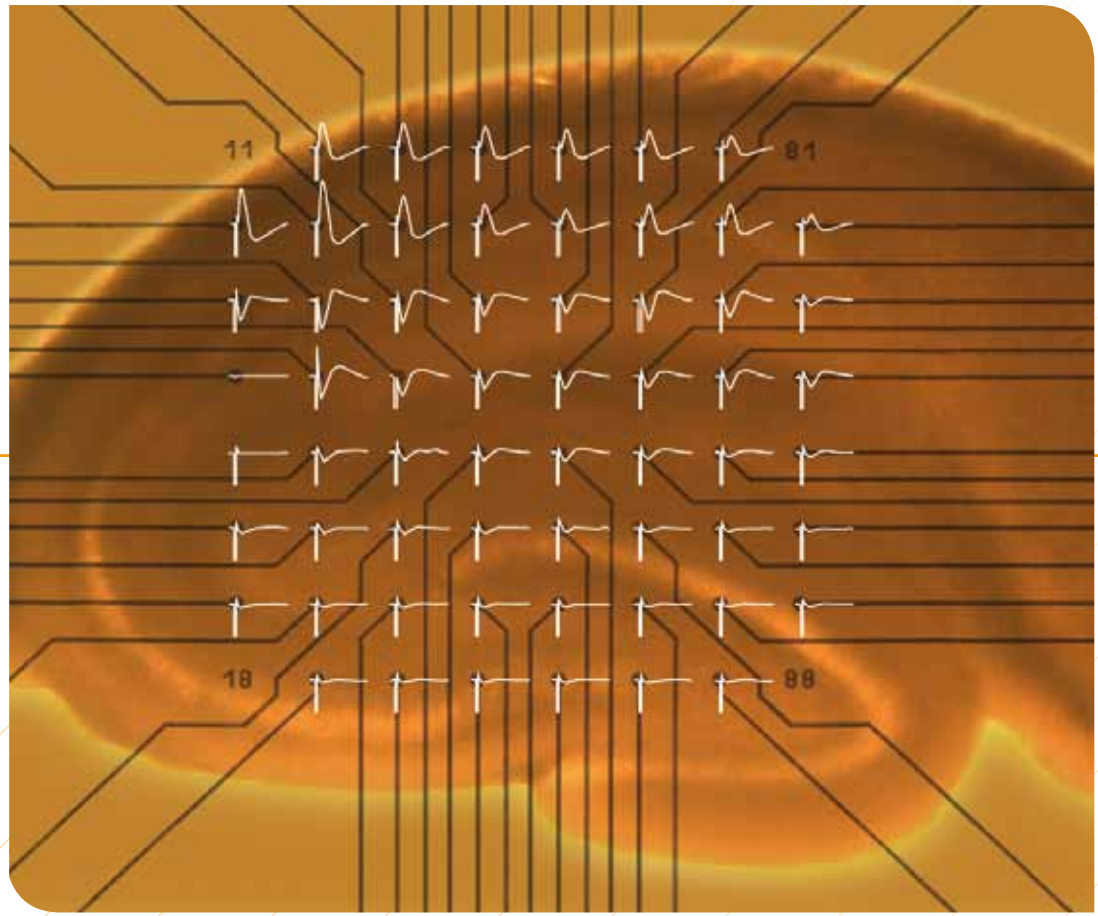
MULTI-ELECTRODE ARRAY
FOR ACUTE BRAIN SLICES RECORDINGS

neuro SERVICE®

WHITE PAPER

CONTENT


11
81
18
88

INTRODUCTION

You are developing new chemical entities and you need the most physiologically relevant environment to profile and optimize your compounds (Structure-Activity Relationships). In addition, you are very concerned with rapid turnaround between biological results and chemical optimization. You want to go beyond classic biochemical or cell-based approaches since you need assays that will integrate the whole complexity of the final target: Central Nervous System (CNS).

Or you want to understand the nature of CNS side-effects for a series of compounds and you need this information as early as possible in the decision-making process. You would like to perform these assays in a highly physiological environment, but for time, cost and compound availability reasons, you have a preference for *in vitro* assays.

Or you have engineered transgenic mice or rats and you need to validate a CNS deficit (or a CNS gain of function) rapidly. At the same time, you might not be aware of the possibility of phenotypical screening based on *in vitro* electrophysiological recordings of acute brain slices prepared from your engineered rodents, with as few as 5 animals per genotype.

Fortunately, there is an especially well-designed way to profile your compounds, to make sure of their safety and phenotype your transgenic animals under completely physiological conditions: acute rodent brain slices recording. These slices can be prepared from many different brain regions and maintained *in vitro* with all neurons and glial cells in place and connected to each other as they would be *in vivo*. All receptors, channels, enzymes are those natives to the tissue with all signaling and regulating pathways remaining functional. This is a true piece of living brain!

Multi-point recordings of acute brain slices with Multi-Electrode Arrays (MEA) are an ideal way to perform functional pharmacology. MEA recordings provide mid-throughput, high quality and highly sensitive assays that are perfectly suited to address your pharmacological needs.

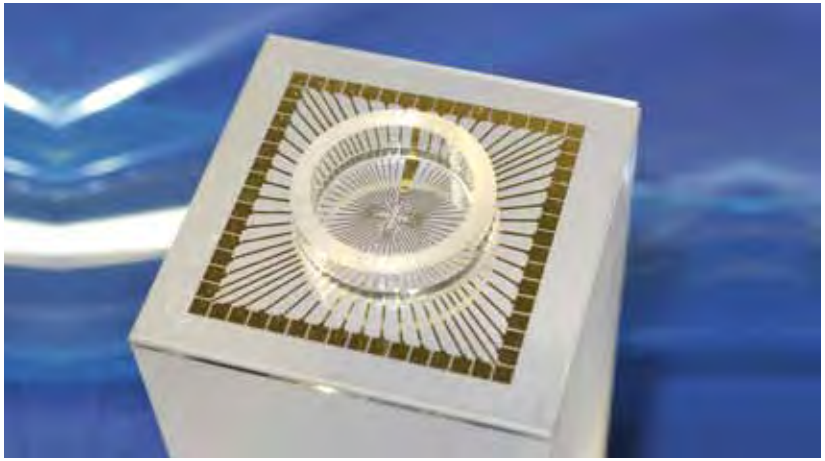
The aim of this White Paper is to illustrate the broad applicability of *in vitro* electrophysiological assays based on the Multi-Electrode Array recording and answer a few basic questions. What is the Multi-Electrode Array technique and what are its benefits? Why should you choose this technique to address the specific questions you may have in your research programs?

1 → What is a Multi-Electrode Array (MEA)?

A Multi-Electrode Array is an array made of microscopic metal electrodes (10-30 µm of diameter) distributed on a small surface area (~0.8-6 mm^2). They are either regularly distributed or they can be arranged to match closely the spatial organization of the brain region investigated. These small electrodes (coated with an inert and bio-compatible metal) are used for recording electrical signals related to neuronal activities within the slice.

These signals can be either action potentials (individual spikes or population spikes) or changes in neuronal membrane potentials resulting from synchronous excitatory and/or inhibitory synaptic transmission. In brain structures such as the hippocampus, cortex and cerebellum, neurons are organized in well-known layers. Thus, one or two MEA electrodes can be used to stimulate a group of neurons and the corresponding "responses" of connected neurons can be recorded by another group of electrodes, a few hundred µm or mm distant from the stimulating point. In this case, Excitatory Post Synaptic Potentials (EPSP) can be recorded since groups of neurons from a defined area typically display synchronous and reproducible activities in response to a single stimulation.

The MEA containing the brain slice is settled in an amplifier headstage (computer driven). A stimulus generator allows stimulating with one MEA electrode (against the ground = GND), or between two MEA electrodes. The oxygenated saline solution is driven by a peristaltic pump and continuously maintained at the expected temperature thanks to a heated-perfusion cannula and a Peltier element located just below the MEA.



> **Figure 1A:**
A standard Multi-Electrode Array (5x5cm) with a 8x8 squared electrodes layout (electrodes spacing 200 µm). The MEA chamber has a ~1 mL volume.

> **Figure 1B:**
A MEA positioned into the amplifier headstage of a compact acquisition system. Continuous slice perfusion and oxygenation is allowed by "in" and "out" tubings (~3 $mL.min^{-1}$).

2 → What kinds of signals are recorded with MEA?

Neurons are excitable cells that generate and propagate action potentials. Neurons connect and communicate with many others through synaptic transmission or direct gap-junction coupling. Action potentials and synaptic transmission involve ion channels opening with associated micro-electrical signals (millivolts fluctuations of their membrane potential). These signals can not only be measured at the single cell level but also at the neuronal network level.

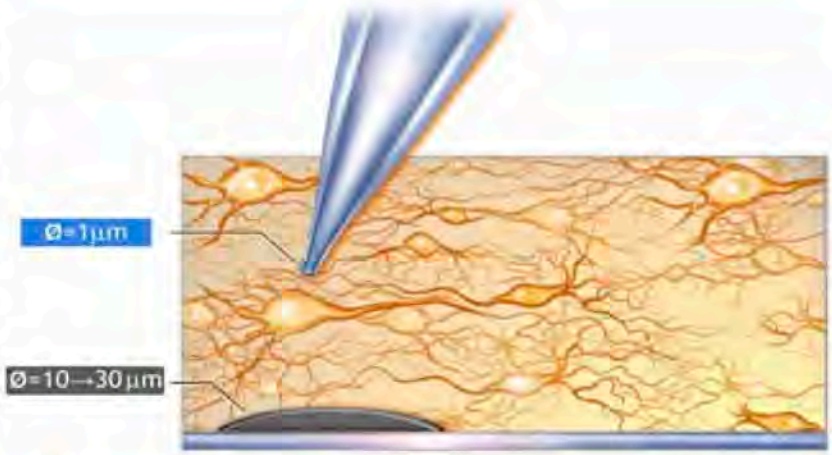
All neuronal signals depend on the underlying metabolic activity of the cell and are constantly regulated by biochemical and signaling pathways. Modifications to these pathways lead to changes in neuronally-associated electrical activities. These electrical signals are consequently an excellent readout to detect the effects of various compounds and/or their potential toxicity on neuronal networks at the second, minute or hour time-scale.

3 → Are there differences between classic glass electrodes and MEA electrodes for extracellular recordings?

The key difference is size. The tip of a glass electrode is very small (~ 1 µm of diameter) whereas a MEA electrode is much larger (10-30 µm of diameter). The glass electrode is carefully positioned very closely to a single neuron within the slice and, as a consequence, discretely picks up only membrane potential fluctuations (in the millisecond range) of this single neuron (i.e. changes in electrical charges at its membrane surface).

On the other hand, MEA electrodes are arranged on a horizontal surface and the brain slice is placed over these electrodes. Like glass electrodes, MEA electrodes also record extracellular signals that correspond to transient changes of neuronal membrane potentials. Because of its size (10-30 µm), and because of its greater distance to the layer of active neurons, one MEA electrode picks up and averages signals generated by the multiple neurons surrounding it.

Thus, glass and MEA electrode signals are not completely equivalent, the signal at a MEA electrode being more integrative than that of a glass electrode.



> **Figure 2:**
Detail of a brain slice recorded with a classical glass electrode (top) and a MEA electrode (bottom). Note the large difference in the electrode sizes.

Clearly the ideal configuration would be to record from every single neuron in a given slice with the ability to integrate all of the individual signals to provide a precise overview of the behavior of the entire neuronal network. This approach has already been attempted with multiple and parallel patch-clamp recordings and with optical (dual photons) recordings. Unfortunately, such technical approaches only provide partial results and are not compatible, regarding time and cost, with the testing of multiple compounds.

To make an analogy with the musical field, imagine the sound recording of a large symphonic orchestra. Theoretically, the sound engineer could place a microphone in front of each instrument and "mix" up to 80-100 individual tracks to "reproduce" the sound of the orchestra. Even with this arrangement, however, the mixing could never recreate the "color" and the global sound of the whole orchestra. Instead, sound engineers typically place a few microphones above groups of instruments to capture this quality. MEA electrodes can be compared to these few microphones placed over the symphonic orchestra (the brain slice). They allow recording the overall "sound" of the neuronal network by integrating the complexity of many signals coming from groups of neurons (the instruments).

4 → What are the advantages of recording brain slices with MEA electrodes?

Your compound can be evaluated *in vitro* under the most physiologically relevant conditions.
As mentioned in the introduction, it is possible to prepare and keep alive rodent brain slices (250-500 µm of thickness). These slices can be prepared from many different brain regions with all neurons and glial cells in place and connected to each other, as they are *in vivo*. In addition, all receptors, channels, enzymes, are the native ones with all signaling and regulating pathways being functional.

MEA recordings increase the robustness of the data.
The ability to record multiple evoked-responses in parallel at different electrodes within a single slice and within the same region of interest increases the reliability of the data and their subsequent statistical processing.

MEA recordings performed in a single brain slice provide an exceptional macroscopic view of neuronal networks.
Region-specific effects can be readily observed over the MEA electrode surface area. As an example, layer-specific activities can be resolved into stratified structures such as hippocampus or cortex slices.

MEA recordings can facilitate the screening of a series of compounds in parallel, with a remarkably quick turnaround (mid-throughput screening).
MEA recordings constitute a well-suited technique for functional series of compound testing as they are much faster than classical glass electrode recordings and as they can be standardized.

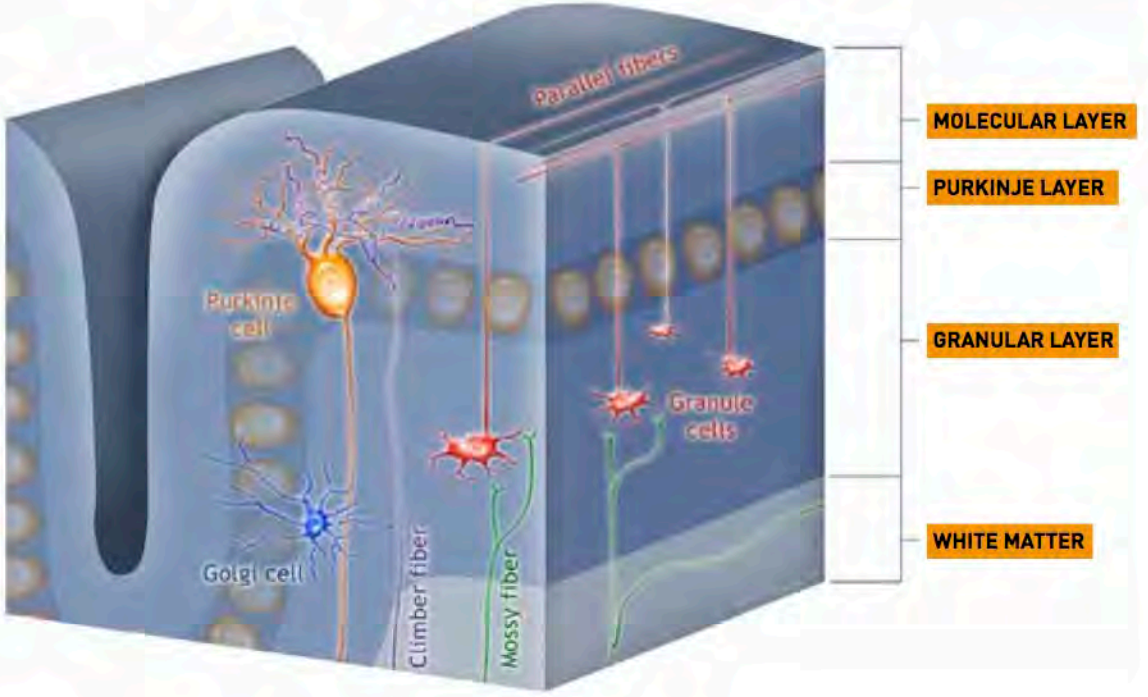
5 → What is the difference between the recording of spontaneous versus evoked activities with MEA electrodes?

Spontaneous activity:

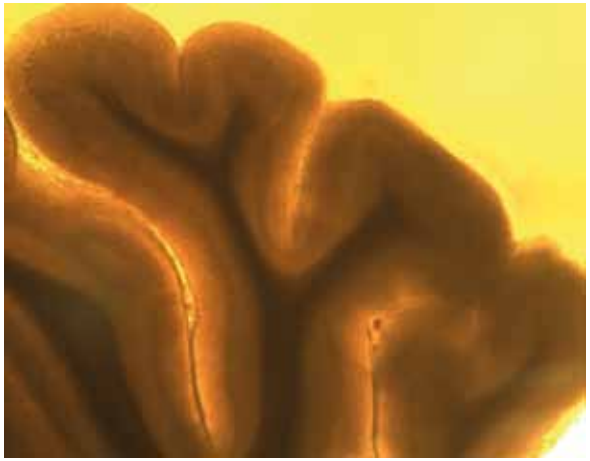
When used as a passive recording system, MEA electrodes can pick up action potentials of spontaneously firing neurons, which is the most basic signaling activity of living neurons.

The MEA electrode can record either the preponderant action potential of a single (large) neuron or the superimposition of a few action potentials from different neurons (which can fire at different frequencies). Over a short period of time, neuronal firing can be stochastic or very regular (when they emerge from pacemaker neurons).

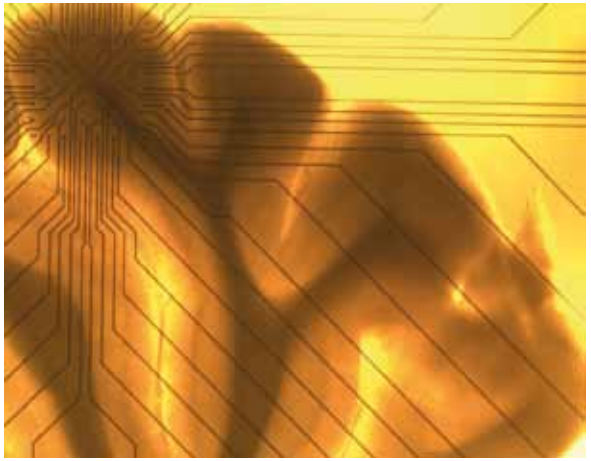
However, in both cases, it is possible to evaluate a compound's effect on neuronal firing by averaging firing over a sufficiently long period of time. Ten minutes are often enough, for instance, to determine a mean firing frequency.



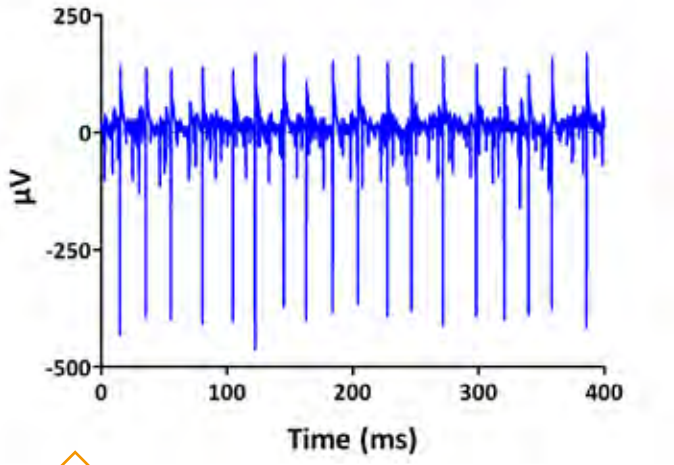
> **Figure 3A:**
3D view of the cerebellum network organization. Note the large neuritic tree of Purkinje neurons.



> **Figure 3B:**
Transverse adult rat cerebellum slice captured with an optic microscope (x40).



> **Figure 3C:**
Transverse adult rat cerebellum slice over a MEA with 100 µm-distant electrodes.

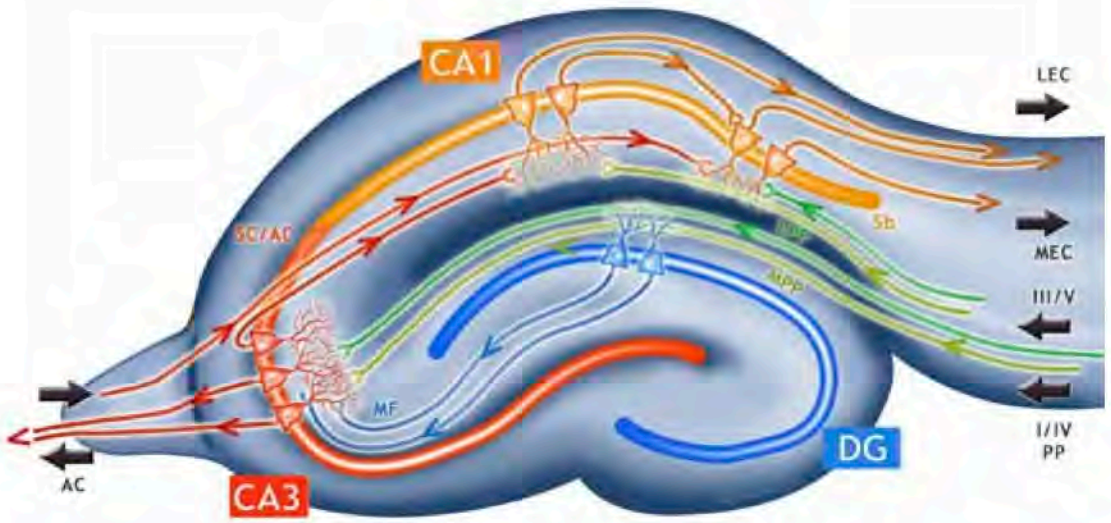


> **Figure 3D:**
Spontaneous firing activities of a few Purkinje neurons recorded at a single MEA electrode in a rat cerebellar slice.

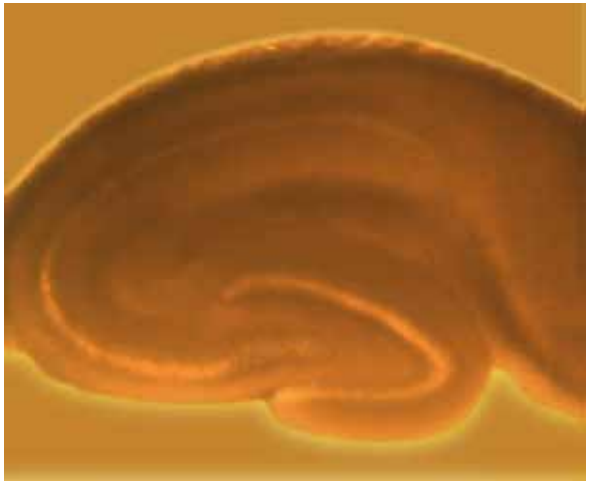
Evoked activity:

When groups of axons are stimulated by a MEA electrode, they fire synchronously and propagate action potentials until their neuronal endings (synaptic boutons). Then, synaptic transmission occurs almost synchronously in one restricted region of the slice containing those synaptic endings.

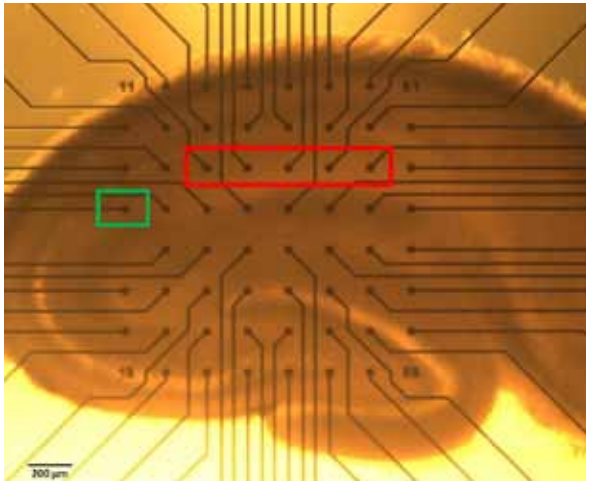
Synaptic transmission is a very fast phenomenon (< 1 ms), but the spatial and temporal sum of many individual transmissions leads to an overall "field potential" with an amplitude of a few 100 microvolts and with a time-course over a few milliseconds. Such a signal is sensitive to drugs that are able to block action potential propagation (such as Tetrodotoxin) or synaptic transmission (such as NBQX). Within the hippocampus, cortex or cerebellum, field potentials result mainly from excitatory transmission mediated by glutamate, the brain's main excitatory neurotransmitter. Counterbalancing that excitatory transmission, there is an inhibitory transmission mediated by the neurotransmitters GABA and glycine.



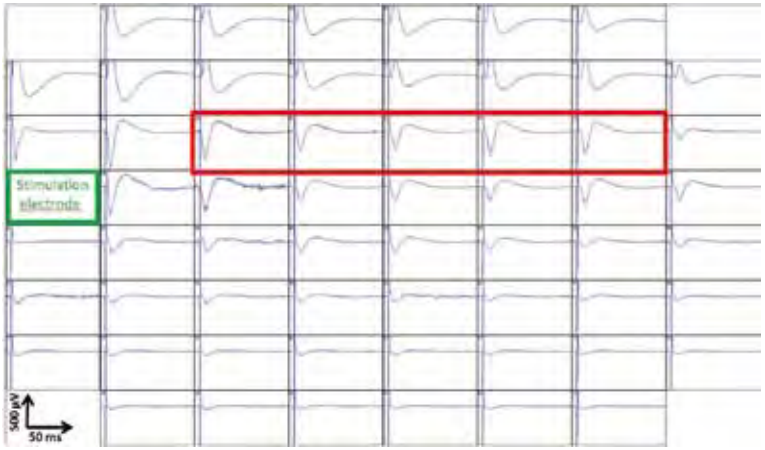
> **Figure 4A:**
Scheme of the hippocampal networks: synapses between Lateral Perforant Path (LPP)/Medial Perforant Path (MPP) fibers (in dark and light green, respectively) and Dentate Gyrus (DG) neurons (in blue); synapses between DG neurons (in blue) and CA3 neurons (in red); and synapses between CA3 neurons (in red) and CA1 neurons (in orange).



> **Figure 4B:**
Transverse adult rat hippocampal slice captured with an optic microscope (x40).



> **Figure 4C:**
Transverse adult rat hippocampal slice over a MEA with 200 µm-distant electrodes. Stimulation and recorded electrodes are surrounded by green and red squares, respectively.



> **Figure 4D:**
Evoked-responses recorded by the MEA electrodes shown in Figure 4C. Each window corresponds to signals recorded by one of the 60 MEA electrodes. The stimulation electrode (at the CA3/CA1 border) is indicated by a green square. Evoked-responses of interest (Excitatory Field Potentials), in the CA1 region, are surrounded by a red square.

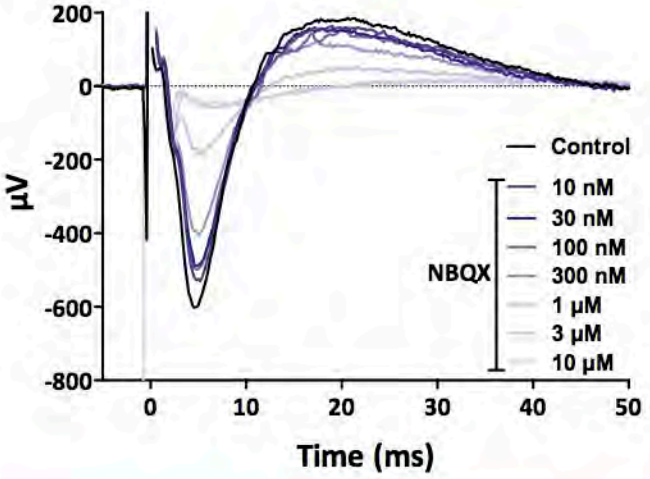
6 → How can MEA recordings be used to investigate the effect(s) of compound(s) and how precise are these measurements?

Dedicated protocols performed in given regions of specific brain slices can be used to address the effect of a compound on a number of parameters (neurotransmission, neurotransmission plasticity processes, neurons' intrinsic properties) or to investigate compound's specific cellular targets and/or Mechanisms Of Action (MOA).

When a compound's clear effect is observed with MEA recordings, further investigations can be performed to determine its pharmacological profile. The effect can be investigated over a wide concentration range with the objective to determine the "Half-Effective Concentration" (EC_{50}) or "Half-Effective Inhibitory Concentration" (IC_{50}). In addition, the effect of allosteric modulators can be investigated on dose-response or dose-inhibition protocols to illustrate their indirect positive or negative activities.

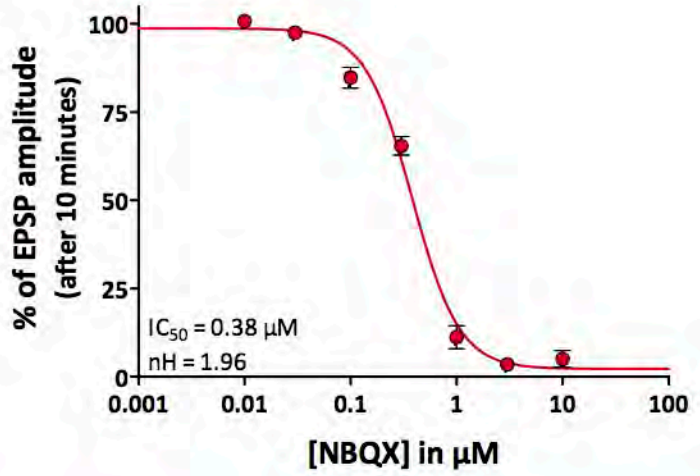
A few experimental measurements are presented to further illustrate the high-precision of pharmacological profile determination that can be performed with MEA recordings.

A first illustration of a compound's direct effect on glutamatergic synaptic transmission is presented in **Figures 5A** and **5B**. The dose-inhibition effect of NBQX (a selective AMPA/Kainate receptor antagonist) on the EPSP amplitude is illustrated for increasing concentrations (**Figure 5A**). Plot of the % of EPSP amplitude as a function of the NBQX concentration allows determining its apparent IC_{50} (fits with the empirical Hill equation; **Figure 5B**).



> **Figure 5A:**

Example of EPSP traces recorded at a single MEA electrode in the CA1 region of an adult rat hippocampal slice in control and after successive 10-minute exposures to increasing NBQX concentrations.

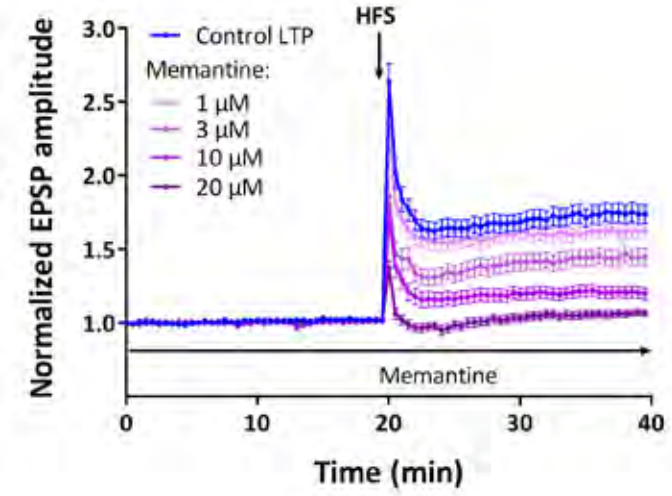


> **Figure 5B:**

Plot of the % of EPSP amplitude as a function of the NBQX concentration on a semi-logarithmic scale. Data points are fitted with an empirical Hill equation yielding an apparent IC_{50} of 0.38 µM.

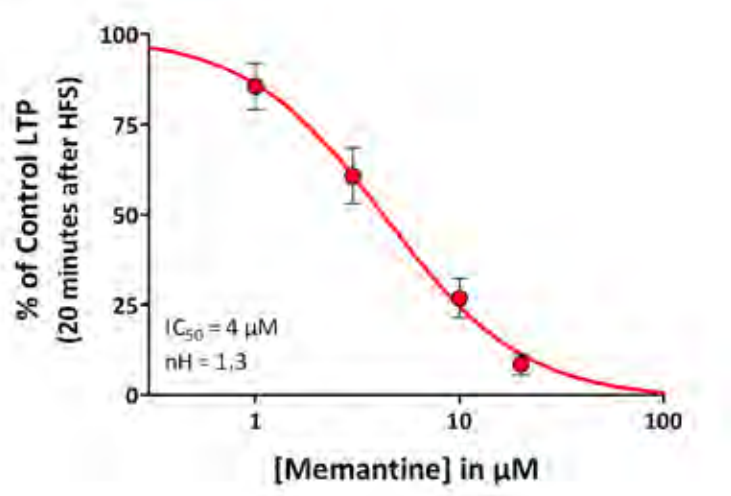
A second example illustrates the dose-dependent effect of Memantine (EBIXA®, a non-competitive inhibitor of NMDA receptors) on Long-Term Potentiation (LTP), a well-recognized paradigm for long-term synaptic plasticity mechanisms.

Following a 20-minute period along which basal synaptic transmission is recorded, a High-Frequency Stimulation (HFS, 100 Hz) is applied and basal synaptic transmission is recorded over another 20-minute period. Very briefly, HFS triggers NMDA receptor activation and many downstream signaling pathways resulting in the potentiation of synaptic transmission. Block of NMDA receptors by Memantine strongly inhibits LTP induction, as illustrated in **Figure 6A**. Plot of the LTP amplitude as a function of the Memantine concentration allows determining an apparent Memantine IC_{50} on LTP (4 µM) which is in accordance with previous values obtained with other electrophysiological techniques and protocols.



> **Figure 6A:**

100 Hz LTP measurement at CA3/CA1 Schaeffer Collaterals (SC) in an adult rat hippocampal slice. LTP was triggered in control slices or in pre-incubated slices with different Memantine concentrations.

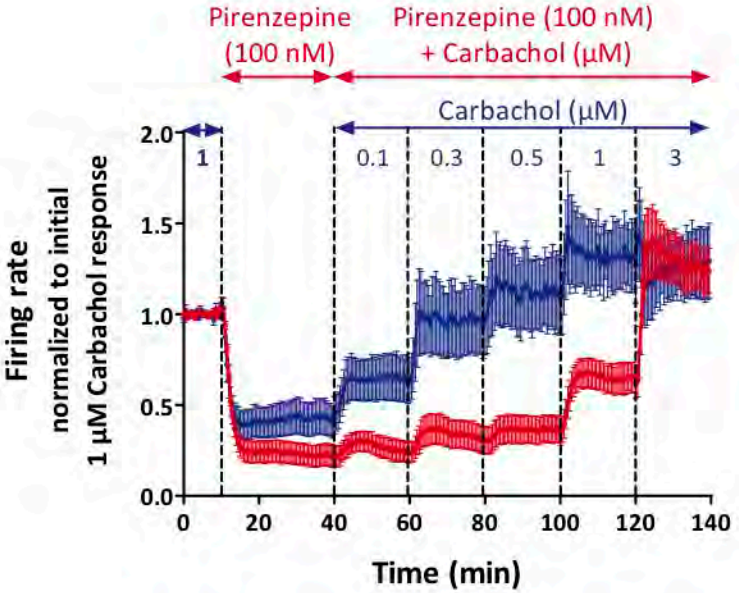


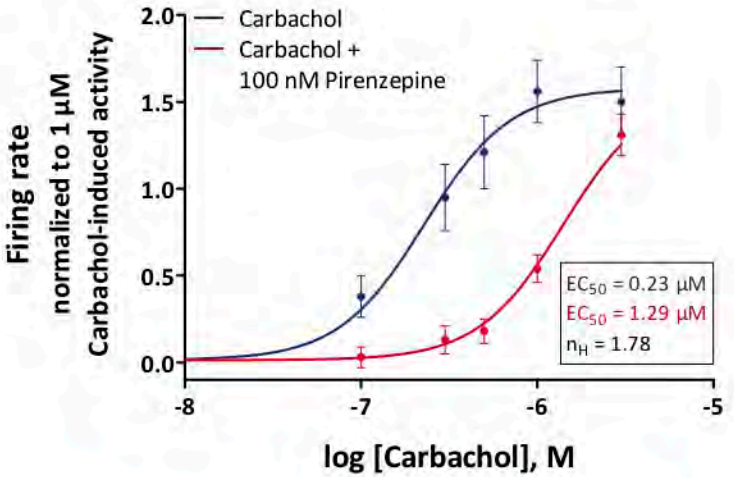
> **Figure 6B:**

LTP dose-inhibition by Memantine. The LTP amplitude normalized to 100% is plotted as a function of the Memantine concentration on a semi-logarithmic scale. Data points are fitted with an empirical Hill equation.

The neuronal spiking activity can be used to investigate the pharmacological profile of compounds. As an illustration, the effect of Carbachol on CA1 neurons firing is presented in **Figure 7A**. Carbachol triggers a dose-dependent increase of CA1 neurons firing through M1 muscarinic AcetylCholine Receptors (M1 mAChR) activation as presented in **Figures 7A** and **B**.

When the same protocol is repeated in the presence of Pirenzepine (a competitive M1 mAChR antagonist), a rightward shift of the Carbachol dose-response relationship is observed, which is consistent with a competitive antagonist's MOA (see **Figure 7B**).





> **Figure 7A:**

CA1 neurons firing activity under increasing concentrations of Carbachol alone (blue) or in combination with Pirenzepine (red). Firing activities are normalized to initial Carbachol-induced firing rate.

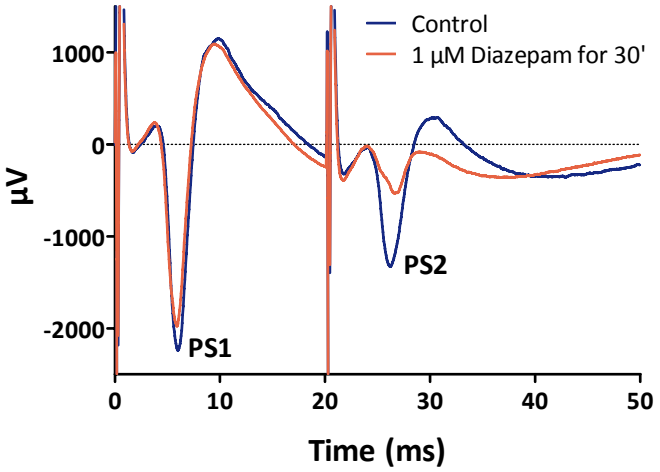
> **Figure 7B:**

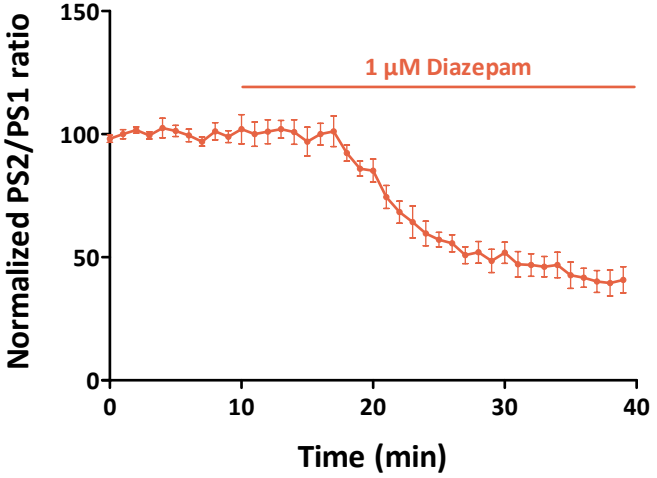
Carbachol dose-response curves on CA1 neurons firing in control conditions (blue) and in the presence of Pirenzepine (red). The normalized firing activity is plotted as a function of the Carbachol concentration. Data points are fitted with the empirical Hill equation.

MEA recordings are also sufficiently refined and sensitive to reveal and document the properties of allosteric modulators. **Figures 8A, 8B** and **8C** present the effect of the $GABA_A$ receptor Positive Allosteric Modulator (PAM) Diazepam. Paired-pulse inhibition experiments allow revealing the $GABA_A$-mediated tonic inhibition of evoked-responses.

This $GABA_A$-mediated inhibition is further revealed by its sensitivity to Diazepam. With paired-stimulations separated by only 20 ms, the GABAergic interneurons-driven inhibition is still activated when the second stimulation occurs.

As a consequence, the response to the second stimulation (second Population Spike, PS2) is of lower amplitude than the one triggered by the first stimulation (Population Spike 1, PS1; see **Figure 8A**). As summarized in **Figure 8C**, $GABA_A$ PAMs enhance $GABA_A$ receptors activity and consequently, the paired-pulse inhibition (resulting in a decrease of PS2/PS1 ratio). On the opposite, a $GABA_A$ antagonist, namely Picrotoxin, abolishes the paired-pulse inhibition (resulting in an increase of PS2/PS1 ratio).



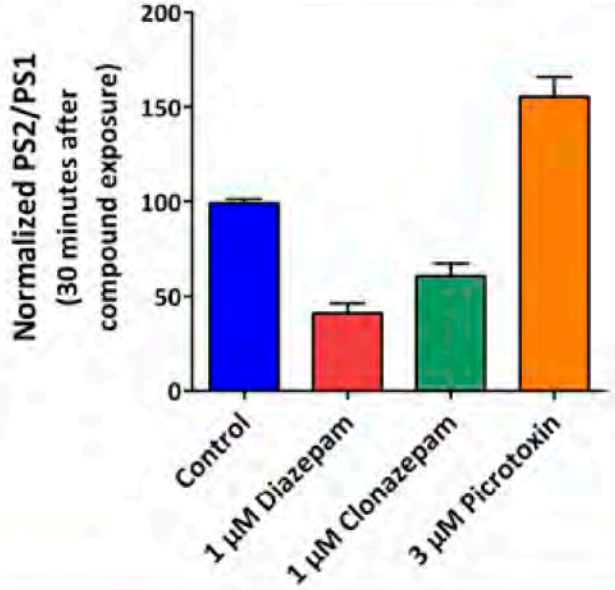


> **Figure 8A:**

Paired-Population Spikes are recorded at SC synapses before and after exposure to 1 µM Diazepam (paired-stimuli of equal intensities). Note the decrease of PS2 in the presence of Diazepam. Positive allosteric modulation of inhibitory $GABA_A$ receptors results in a stronger inhibition of PS2.

> **Figure 8B:**

Time-course of the Diazepam effect on PS Paired-Pulse Ratio (PPR). PS2/PS1 ratio (normalized to 100%) is expressed as a function of time. Note that the Diazepam effect slowly occurs and reaches a steady state after 30 minutes of exposure.



> **Figure 8C:**

Comparison of classical PAMs and a competitive antagonist of $GABA_A$ receptors. $GABA_A$ PAMs, Diazepam and Clonazepam, enhance the PPI (observed as a PPR decrease), whereas the $GABA_A$ antagonist Picrotoxin, blocks the PPI (measured as an increase of the PPR).

7 → Functional screening of series of compounds in brain slices with MEA recordings.

MEA recordings are of particular interest for screening small chemical libraries, for lead selection or lead optimization. Most of the time, a reference pharmacological compound is used to validate the measurements and provide a reference activity.

NEUROSERVICE has developed and validated many robust MEA-standardized assays that can be used individually or in parallel to screen series of chemicals. Such repetitive assays are performed over weeks, months or years within the framework of very well-defined conditions (protocols and Standard Operating Procedures) that guarantee the reproducibility and robustness of data.

In terms of timing, the screening of a series of compounds (up to 100 slices per week) can often be accomplished over only a few days or weeks, providing a very dynamic interaction between biologists and chemists.

CONCLUSION

As illustrated, several properties of neurons and neuronal networks can be investigated in acute brain slices with MEA recordings. This electrophysiological technique is very efficient in terms of slices turnaround and compounds' testing. In addition, very precise pharmacological effects and profiles can be determined while monitoring different parameters.

Acute brain slice recordings with MEA can provide answers to most of the questions you may have:

- Investigate or confirm your compounds' targets and/or MOA, thanks to a wide panel of tests within **a fully physiological context.**

- Phenotype your transgenic mice/rat for a wide range of neuronal properties and with **a minimal number of animals.**

- Clarify compounds' safety with **rapid turnaround** and **reasonable cost** with only **a small quantity of compound.**

Reap the benefits of high-content screening through MEA recordings!

You will not miss any of your compound's effects with MEA recordings!

NEUROSERVICE is a customer-driven Contract Research Organization (CRO), the source for high scientific expertise (in the fields of electrophysiological techniques, neuropharmacology, neurophysiology and neurotoxicology) and state-of-the-art skills. NEUROSERVICE already provides high-quality data and R&D support to top-tier Pharmaceutical companies, Biotech companies and Foundations worldwide.

NEUROSERVICE offers, at the same time, screening assays based on acute brain slice recordings and a "package of assays" designed to investigate and screen possible CNS side-effects.

In addition, NEUROSERVICE's scientists have demonstrated skills to design and perform your protocols of interest as well as to solve pharmacological, physiological or technical issues on a daily basis.

NEUROSERVICE provides very flexible study plans (modifications and/or adaptations of protocols can be done at any step) and frequent releases of analyzed data, so that you can readily track progress for your Research Project.

NEUROSERVICE also promotes very close working relationships, so that you can contact us and discuss results or new projects at any time. The study reports are released with the most complete analyzed results and, if needed, we can provide you with the complete raw data.

As a CRO, we are 100% transparent since our objective is to provide top-quality data and analyses as well as strong scientific expertise.

NEUROSERVICE remains at your disposal for any further questions:

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Contact us and we will discuss with you, depending on your research programs, about the benefits of *in vitro* electrophysiological assays based on the MEA technique!

AUTHORS

Bruno BUISSON, PhD
Founder •
General Manager
& Chief Scientist

Bruno is Founder, General Manager & Chief Scientist at NEUROSERVICE. Trained as a biochemist, neurophysiologist and pharmacologist, Bruno has more than 20 years of experience in electrophysiology (Patch-Clamp and Multi-Electrode Arrays) and receptors pharmacology. Throughout a 6-year period, as Head of Pharmacology & Toxicology at TROPHOS (a French biotech), he enriched his experience in Drug Discovery programs targeting Central Nervous System pathologies.

He is the co-author of 2 patented compounds that have been brought to Phase II in less than 5 years (for CNS and cardio-vascular diseases). Bruno has supervised the development and the industrialization of many electrophysiological-based assays for CNS Drug Discovery, rising NEUROSERVICE as the world-leading company in its field of activity.

He attended the Ecole Normale Supérieure (Cachan) and received a PhD in Biochemistry and Neurosciences from the University of Montpellier (France). He was a postdoctoral fellow in the laboratory of Daniel Bertrand at the University of Geneva (Switzerland).

Esther-Marie STEIDL, MsSc
Co-Founder •
Head of the Multi-Electrode
Array Laboratory

Esther-Marie is Co-Founder and Head of MEA Laboratory at NEUROSERVICE. Esther-Marie has managed NEUROSERVICE's MEA Laboratory since its inception.

Over an 8-year experience with this technique, she has developed and validated dozens of pharmacological and screening assays. Working in close collaboration with scientists from many big pharmaceutical and biotech companies, she has gained a unique experience in drug testing on acute slices with MEA.

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GLOSSARY

AMPA.....α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

CNS.....Central Nervous System

DG.....Dentate Gyrus

EPSP.....Excitatory Post-Synaptic Potential

GABA.....γ-AminoButyric Acid

$GABA_A$..... type A GABA receptor

GND.....Ground

HFS.....High Frequency Stimulation

LPP.....Lateral Perforant Path

LTP.....Long-Term Potentiation

M1 mAChR.....M1 muscarinic AcetylCholine Receptor

MEA.....Multi-Electrode Array

MOA.....Mechanism Of Action

MPP.....Medial Perforant Path

NBQX.....6-nitro-7-sulphamobenzoquinoxaline-2,3-dione

NMDA.....N-Methyl-D-Aspartic acid

PAM.....Positive Allosteric Modulator

PPI.....Paired-Pulse Inhibition

PPR.....Paired-Pulse Ratio

PS.....Population Spike

SC.....Schaeffer Collaterals

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