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MEA Application Note:
Circadian Rhythm Suprachiasmatic Nucleus Neurons & Organotypic Cultures

MEA

Imprint

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A major part of this information is based on the instructions provided by the laboratory of Dr. Christopher Klisch and Prof. Hilmar Meissl. We are very thankful for their support.

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About this Application Note

The intention of the MEA Application Notes is to show users how to set up real experiments with the MEA-System based on typical applications that are used worldwide.

The documents have been written by or with the support of experienced MEA users who like to share their experience with new users.

This application note includes a complete protocol for the isolation and cultivation of suprachiasmatic nucleus (SCN) neurons, suggestions for long-term cultures, suggestions for MEA-System configurations, example data, and references.

Biological Materials

15 Postnatal rats (Wistar Kyoto or Sprague Dawley) day 1 to 7.

The most commonly used species are rat, mice, or hamster. All three species reveal circadian periodicity in the firing rate of SCN neurons maintained in cell culture.

It is recommended to use neurons isolated from animals aged between the postnatal day 1 to 7 to obtain a maximum rate of surviving neurons. Neurons from young animal are less susceptible to damage during the dissociation procedure.

The SCN already exhibits many of its mature properties at this age. The anatomical region of the SCN can be clearly recognized.

Technical Equipment

- MEA-System (with amplifier and data acquisition, see "Suggested MEA-System")
- MEAs (microelectrode arrays), for example, standard MEAs with 30 µm electrodes
- Sterile workbench
- Incubator set to 35 °C, 65 % relative humidity, 9 % O_2, 5 % CO_2 or 37 °C, 100 % humidity, 5 % CO_2
- Water bath at 37 °C
- Tissue chopper
- Stereo microscope
- Inverted or upright microscope
- Micropipettes and pipette tips (20 µl and 1000 µl)
- 15 ml BD Falcon tubes
- 70 µm nylon mesh cell strainer (BD Falcon)
- Sharp forceps
- Curved forceps
- Small scissors
- 20 gauge needle (1.5 – 1.6 mm)
- Teflon membranes (ALA Scientific Instruments Inc.)

Chemicals

- Trypsin inhibitor from chicken egg white (Sigma-Aldrich, Inc., T9253)
- Papain 30.8 mg/ml, 28 U/mg (Worthington Biochemical Corporation, LS003126)
- HEPES buffer (Sigma-Aldrich, Inc., H3375)
- Earl's Balanced Salt Solution (EBSS), stored at 4 °C (Gibco/Invitrogen, 24010-43)
- DMEM Dulbecco's Modified Eagle Media (DMEM) / F12 (Gibco/Invitrogen, 21331-20)
- Glucose D (+) (Merck KGaA,108337)
- L-Cystein (Sigma-Aldrich, Inc., C-6852)
- Bovine serum albumin (BSA), Fraction V, cell culture tested (Sigma-Aldrich, Inc., A-9418)
- EDTA, 250 mM

Media

EBSS-7

- 50 ml EBSS stored at 4 °C
- 560 µl 3 M Glucose D (+) >> 7 g/l
- 500 µl 1 M HEPES >> 10 mM

100 mM Cystein Solution

- 17 mg / ml cystein in distilled water

Enzyme solution

→ Before use, activate the solution by 37 °C for 20 min, followed by sterile filtration.

- 2.5 ml EBSS-7
- 5 µl 250 mM EDTA >> 0.5 mM
- 25 µl 100 mM Cystein solution >> 1 mM
- ~50 µl Papain >> 20 U/ml

EBSS-BSA-TI-1

- 3 ml EBSS-7
- 3 mg Trypsin inhibitor >> 0.1 %
- 30 mg BSA >> 1 %

EBSS-BSA-TI-0.1

- 0.5 ml EBSS-BSA-TI-1
- 4.5 ml EBSS-7

Methods

MEA Coating

Depending on the type of selected MEA, various coatings may be applied. We recommend to coat standard MEAs with Poly-D-Lysine + Fibronectin for this application. Suggestions for coating methods can be found in the MEA manual available in the "**Download" section** of the MCS web site.

Dissection

Before the dissection, the animals are typically kept in a daily light-dark cycle of 12 h.

In contrast to acute slices, you cannot predict the phases of the circadian rhythm in cell cultures or organotypic slices. You will observe free running rhythms because of the absence of entraining mechanisms.

1. Decapitate the animal.
2. Open the skull carefully with a scissor; cut the optic nerves with a fine scissor and remove the brain.
3. Prepare coronal sections of the hypothalamus (with a thickness of approximately 500 µm) using a tissue chopper, a vibratome, or by hand with a razor blade. Use the optic chiasm and the third ventricle as landmarks. If you cut directly in front of the optic chiasm, and perform the second cut caudally to the chiasm, the SCN is obtained in a single slice.
4. Punch the SCN using a 20-gauge needle. Up to two of those fragments can be obtained per animal. Collect all fragments in ice-cold EBSS-7 buffer in a Falcon tube.

Enzymatic Digestion

1. Remove the buffer solution and replace it with 2 – 3 ml enzyme solution pre-warmed at 37 °C.
2. Dissociate the hypothalamus fragments with the enzyme solution at 37 °C for 15 - 30 min. Gently triturate each 10 min by passing the preparation five times through the 0.78 mm wide opening of a 1000 µl pipette tip. The majority of cells should now be in suspension.
3. Centrifuge at 300 g and 4 °C for 10 min.
4. Discard the supernatant and resuspend the cells in approximately 0.5 ml TI-0.1.
5. Pipette the cell suspension onto 1 ml of TI-1, resulting in a concentration gradient of trypsine inhibitor in the tube.
6. Centrifuge at 100 g and 4 °C for 15 min.
7. Discard the supernatant and resuspend the pellet in ice-cold DMEM/F12.
8. Centrifuge at 300 g and 4 °C for 7 min.
9. Discard the supernatant. Leave only a little volume of DMEM/F12.

Plating und Culturing the Cells

1. Count the cells under a stereo microscope to determine the cell density by using a Neubauer chamber or an automated cell counter.

2. Plate the cells in a density of 5000 cells per mm^2 or higher (approximately 6 –10 µl of the suspension) onto the recording field of the MEA. Pipette a few drops of DMEM/F12 near the rim of the MEA culture chamber.

1. Incubate for about 60 min in an incubator. To avoid drying out the culture, place the MEA in a big Petri dish with lid on.

2. Gently pipette DMEM/F12 onto the cells and fill the culture chamber.

Maintain the cells for 2 – 3 weeks in an incubator at 37 °C with 5 % CO_2 before recording.
Replace the medium about two or three times a week. The culture can be used for several months.

Long-Term Culturing

In order to allow long-term cultivation and recording without a continuous perfusion, Multi Channel Systems MCS GmbH recommends the use of teflon membranes developed by Potter and DeMarse 2001).

The sealed MEA culture chamber with transparent semipermeable membrane is suitable for all MEAs with glass ring. A hydrophobic semipermeable foil from Dupont that is selectively permeable to gases (O_2, CO_2), but not to fluid, keeps your culture clean and sterile, preventing contaminations by airborne pathogens. It also greatly reduces evaporation and thus prevents a dry-out of the culture.

The ALA-MEA-MEM membrane is produced in license by ALA Scientific Instruments Inc. and distributed via the world-wide network of MCS distributors.

Another possibility is to use a MEA culture chamber with lid (available from Multi Channel Systems MCS GmbH), and a suitable perfusion system for a continuous perfusion.

Perfusion and Microdrop Application

You can modify the lid of a MEA culture chamber as follows: Drill small holes into the lid and insert metal cannulas (that can be connected to ground) as a perfusion inlet and outlet. This way, you have a sealed sterile environment.

For driving the perfusion, we suggest the use of a push-pull pump that holds paired syringes that can cycle continuously back and forth in a push-pull action. One syringe is infusing, the other is withdrawing at the same flow rate. This efficiently prevents that any liquid overflows and is spilled onto the amplifier.

For slow flow rates, the heating element integrated into the MEA2100 headstage or into the MEA1060 amplifier is sufficient for controlling the bath temperature. For higher flow rates, the use of a perfusion cannula PH01 with integrated temperature control (from Multi Channel Systems MCS GmbH) is recommended.

Compounds can be locally applied to the cell culture by using a Hamilton syringe, which allows you, depending on the syringe model to inject from 0.1 µl up to several microliters of substance at a time. Typically, these syringes are made from borosilicate glass, and have a carbon fiber or metal plunger.

Sources of supply:

- Syringe pump, infusion-withdrawal (double) single cycle action, product number SP260p from WPI World Precision Instruments, www.wpiinc.com. This pump can hold syringes in the range of 10 µl up to 140 ml, and features flow rates ranging from 0.001 µl/h to 147 ml/min.
- Syringes for microdrop application from Hamilton Company, www.hamiltoncompany.com

Suggested MEA-System

System Configurations

Depending on the throughput and the analysis requirements desired in your laboratory, different system configurations are recommended for the recording from cultured suprachiasmatic nucleus neurons.

- **MEA2100-System**: For the **MEA2100-HS60(2x60)-System**, the same MEAs can be used as for the MEA1060 amplifiers, with the same advantages and disadvantages. In addition, a 120-channel MEA layout is available (120MEA200/30iR) for the **MEA2100-HS120-System**, which provides a larger electrode field with a resolution identical to the 60MEA200/30iR. This might be interesting for a mapping application. The MEA2100-HS60-System allows to operate a maximum of two headstages with up to two 60-channel MEAs each.
- **USB-MEA60-System**: **60-channel** MEA recording system for inverted or upright microscopes. The **temperature controller** TCX regulates the temperature of the MEA and of the perfusion fluid via the **perfusion cannula** PH01. A peristaltic perfusion system PPS2 is provided respectively. A **MEA1060 amplifier** allows recording up to 60-channels from one MEA. This is the standard configuration for low-throughput academic research.

Microelectrode Arrays

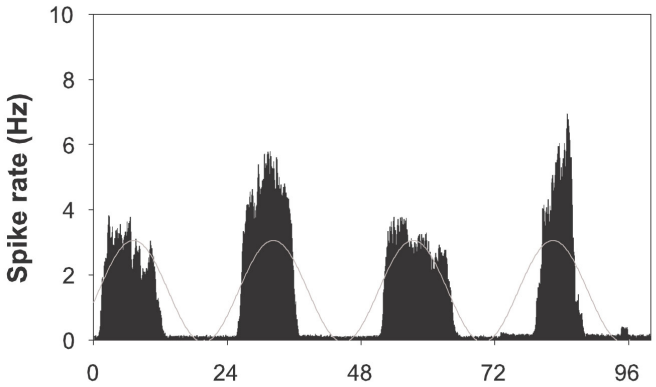
Available MEAs differ in electrode material, diameter, and spacing. For an overview on available MEA types please see MEA manual or the Multi Channel Systems web site (**www.multichannelsystems.com**) or contact your local retailer.

The microfold structures formed by titanium nitride (TiN) result in a large surface area that allows the design of small electrodes with a low impedance and an excellent signal to noise ratio. For recording from cultured neurons, a medium spatial resolution with an electrode diameter of 30 µm and a spacing of 200 µm is generally sufficient.

Recommended MEAs include:

- **60MEA200/30iR-Ti** standard 8 x 8 layout, TiN electrodes for recording and stimulation, with substrate-integrated reference electrode.
- **60ThinMEA200/30i-ITO** for high-resolution imaging and combination with intracellular calcium measurements. ThinMEAs are only 180 µm "thick" and mounted on a robust ceramic carrier. Tracks and contact pads are made of transparent ITO.

Application Example



Circadian firing rhythm of a rat SCN neuron cultured on a standard 60MEA200/30iR-Ti.
It is possible to describe the activity by a cosine function with a period of 25.1 hours.

References

1. E. Tousson, H. Meissl,
Suprachiasmatic nuclei grafts restore the circadian rhythm in the paraventricular nucleus of the hypothalamus, J Neurosci 24, 2983-8 (Mar 24, 2004).

2. M. H. Hastings,E. D. Herzog,
Clock genes, oscillators, and cellular networks in the suprachiasmatic nuclei. J Biol Rhythms. 2004 Oct;19(5):400-13.

3. E. D. Herzog, R. M. Huckfeldt,
Circadian entrainment to temperature, but not light, in the isolated suprachiasmatic nucleus, J Neurophysiol 90, 763-70 (Aug, 2003).

4. E. D. Herzog, S. J. Aton, R. Numano, Y. Sakaki, H. Tei,
Temporal precision in the mammalian circadian system: a reliable clock from less reliable neurons, J Biol Rhythms 19, 35-46 (Feb, 2004).

5. E. D. Herzog, S. J. Aton, C. S. Colwell, A. J. Harmar, J. Waschek,
Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons, Nat Neurosci. 2005 Mar 6.

6. D. Ren, J.D. Miller,
Primary cell culture of suprachiasmatic nucleus. Brain Res Bull. 2003 Sep 30;61(5):547-53. Review.

7. R. N. Van Gelder, E. D. Herzog, W. J. Schwartz, P. H. Taghert, Circadian rhythms: in the loop at last, Science 300, 1534-5 (Jun 6, 2003).