Isolated nerve tissue systems
The solutions of the in-vitro superfusion release technique result in a system, which is easy to use and can be assembled in a mobile way. It makes possible to get the neurotransmitters with electrical or chemical stimulation from the nerve tissues (for details see How can the system help? section). The system consists of three main units:

1. The central unit with superfusion chamber and pre-heater,
2. Heat-stabilizer and buffer conveyor unit.
COMPONENTS:

1. Central unit with superfusion chamber and preheater

The component elements are placed on a specially designed stand in the following order, going from the top to the bottom:

a) Incubation chamber with carbogen controller,

b) Slice chambers with bubble trap and stimulating electrodes (superfusion unit),

c) Thermal equalizer pigs (pre-heater).

a) Incubation chamber with carbogen controller

The double-walled, 10 ml capacity chamber is connected to the thermo stabilizing unit through the liquid steamed in the outer sheath. The chamber is to keep the tissue slices prepared for the measurement alive carbogenized, and in the appropriate temperature until the measurement starts. The continuity and appropriate condition of the gas necessary for incubation is provided by the wind boiler, the fine adjuster and the atomizer unit.

The unit makes the process of measurement easier and faster.

b) Slice chambers with bubble trap and stimulating electrodes (superfusion unit)

- The slice chambers are situated in the holder. The holders contain the stimulating electrodes. The electrodes are directly connected to the banana socket, which hosts the gold-plated electro stimulating cable.

- The slice chamber can be fitted into the sealed slot, which is in the inner surface of the holder. The holder can be closed with the mounting screws after the chamber is inserted. The bubble trap is located at the buffer in point of the lower closing holder.

- The buffer out can be found at the upper closing holder.
The slice chamber is the most important element of the system. So it was designed in a way, which makes it possible for the tissue slice to be put into the chamber the fastest and safest way possible, before the measurement. It is also important that the stimuli, starting the process (electrical or chemical stimulation), can reach the tissue slice with the best efficiency. Besides keeping the buffer liquid in a stable temperature, the continuous and bubble-free flow must also be supported. To ensure these conditions, the chamber is made up from five easily attachable units.

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2. Thermal stabilizer and buffer conveyor unit

The buffer liquid is situated in the thermo stabilized tank of the water heater and circulating thermostat (Circulation waterbath). The inner temperature of the tank can be adjusted between 25-45°C with 0.1°C accuracy. The buffer liquid is delivered to the measuring space through the peristaltic pump. The heat loss, during delivery is replaced by the thermo equalizer pig with outer cloak. The pig is connected to the thermo stabilizer water circulating circle. The heat loss of the buffer liquid, running through the spiral of the pig, is supplied there. The inner temperature of the pig is calibrated to the temperature required in the measuring space.
**ADDITIONAL EQUIPMENTS REQUIRED FOR OPERATION:**

- **CW-02 Circulation water bath**
- **PRO-IM-02 Pelistaltic pump**
- **FC 203 B Fraction collector**
- **EXP-ST-04 Square wave stimulator**

The study of neurotransmitter release and influencing the experiment are essential parts of studying information transfer between nerve cells and other regulatory processes. Neurotransmitter release in in-vitro circumstances can be performed by the electrical or chemical stimulation of nerve tissue dissection. Given the particular sensitivity of the nerve cells, continuous stable supply of oxygen and nutrients must be provided for the dissection the experiment. The closed-system superfusion device is used for this purpose. The peristaltic pump ensures the continuous, pre-heated, carbonized physiological solution supply of the nerve cell dissection, which is placed in the perfusion chamber with minimal dead space. The complete perfusion system includes the electric stimulator, the thermostat, carbogen (95% O₂, 5% CO₂) gas meter and the perfusion chambers. The great advantage of the perfusion chamber is that it is suitable for the examination of electrical field stimulation-, and chemical stimulation-induced release of neurotransmitters. The electrical excitation of the slice are ensured by the platinum electrodes, which are built in the chamber, while chemical stimulation can be achieved by compounds dissolved in perfused liquids, or the chemical excitation substances can be directly delivered to the perfusion chambers. The flow rate can be controlled, it can even be stopped for a given time interval (e.g.: experimental ischemia). The effluent, leaving the chambers contains the radioactive labelled neurotransmitter, which was released from the dissection by stimulation.
In practice, this latter one is virtually any neurotransmitter-, or neuromodulator-type material, which has an isotope labeled version, and the nerve cells in the tissue are able to pick them up, store them, and after a relevant stimuli give them off into the space between the cell. The most commonly used molecules in experiments are the ones with tritium label, but 14C labeled isotopes can also be. In addition to that, the two isotopes can be used simultaneously, which allows us the parallel study of two different transmitter / modulator release. The continuously circulating perfusion liquid in the washes part of the released radioactive neurotransmitter from the space between the cells, and thus appears in the effluent, so it can be measured. The major advantage of the radioactive neurotransmitter release techniques that test results are available within a few hours.

In contrast to the conventional type isotope delivery to tissue (soaking), we developed a more reliable, faster and safer method. For this we have developed the **LU-01 loading unit**, which can be organically integrated into the measuring system.

The essence of the **LU-01 loading unit** is that the tracers (e.g.: isotope) can be introduced in to the tissue from a closed, heat-stabilized liquid chamber by nerve impulses. The tissue is placed in the incubator vessel the same way as it is placed in the perfusion unit of the measuring system. The same applies to the transmission of stimulus current (background stimulation). The outer jacket of the vessel is organically connected to the heating circle of the measuring system, however, carbogen supply and buffersolution input is performed independently.
The system consists of three main components:

1. Central unit with measuring chamber and pre-heater
2. Biopotential amplifier and electrostimulator,
3. Heat stabilizer and buffer conveyor,

Theoretical arrangement:

The in-vitro two-chamber superfusion system is one of the most important members of the MDE GmbH nerve tissue examination equipment. However, the nerve tissues are spatially separated in the split chambers, they remain functionally connected. The two-chamber brain slice superfusion system is the unique combination of the wedge preparation technique, the in-vitro superfusion system and the radioactive neurotransmitter release technique. Its aim is to aid the examination of biopotential responses when the examined nerve tissue is selectively, and there is a simultaneous neurotransmitter release in a different area of the same tissue.
The components of the central unit are placed on a specially designed stand in the following order, going from the top to the bottom:

- Incubation chamber with carbogen controller
- Measuring chambers with bubble trap, spacer disks, and stimulating and measuring electrodes,
- Pre-heater.

**Incubation vessel with carbogen controller**

The 10 ml-capacity, double walled vessel is connected to the heat-stabilizer unit through the liquid circulating in the outer sheath. The vessel is responsible to keep the prepared tissue slices alive, while incubated in a suitable temperature and carbogenized until the measurement starts. The continuity and appropriate condition of the gas necessary for incubation is provided by the wind boiler, the fine adjuster and the atomizer unit

*The unit makes the measurement easier and faster.*

**Measuring unit**

The physiological conditions, which are necessary to keep the tissue alive in the measuring chamber is ensured by the heat-stabilized physiological solution (buffer), which is running through the pre-heater. The optimal fluid level of the chambers is supported by the continuous circulation and extraction of a peristaltic pump. The two chambers are separated by a spacer disk with silicon sealing. The stimulating and measuring electrodes can be found in both chambers, enabling bidirectional measurement and stimulation.

Two measuring nipples are providing the temperature adjustment and continuous monitoring of the chamber liquid, which are suitable to receive thermosensor signals (optional accessories, built in only per request).
Measuring chambers
The open organ vessel can be separated into two with a plexiglass spacer. The spacer divides the vessel into two compartments of 3 ml volume each. In case of the Cortical Wedge the tissue can be positioned in the fluid space (for example) in such a way that the cortical part and the striatal part are separated from one other in the line of the corpus callosum. The separator wall and the inserted tissue are surrounded by high purity silicon grease, so no leakage can occur between the compartments. A peristaltic pump is continuously circulating buffer through the two compartments, however, independently from one other. The injected liquid first enters the small chamber, which is in front of the compartments. This has a double role: on the other hand it removes the bubbles from the hot, oxygenized liquid; on the other hand it decreases the periodic liquid wave, generated by the peristaltic pump. The inlet and outlet are spaced relatively shifted, supporting a cross-liquid flow, which ensures the smallest dead space possible in the chamber.

In case of an open perfusion system, the compound can be directly injected into the chamber, next to the tissue or perfusion can also be stopped (to prevent the leaching of the compound).

Biopotential amplifier (EXT MW-04)
The amplifier was developed for the potential changes sensed by the Ag/AgCl electrodes. The sensors transfer the measured potential changes through the liquid directly connected to the AC/DC inlet amplifier. The amplifier has an internal calibration and offset equalizer. The analog outlet of the amplifier is compatible with any standard AD converter, making it connectable to any chart software in the market.

Technical parameters:
Input resistance: 10 MΩ
Amplification: x100, x1000
Zero offset: +/-10 mVolts, referring to the input
Test circuit: 10 kΩ /250 mV (gain= x100)
Output resistance: 100 Ω
Carrying capacity of Output: 4 mA
Weight: 8 kgs with power supply
Additional equipments required for operation:

- CWB-02 Circulation water bath
- PRO-IM-02 Pelistaltic pump
- FC 203 B Fraction collector
- EXP-ST-04 Square wave stimulator
How can the system help?

The two-chamber in-vitro perfusion system was developed from the combination of two well-known techniques (the wedge evoked potential measurements and the release of neurotransmitter release). Its purpose is to measure the potentials evoked by selective stimulation (electrical or chemical) of the examined tissue and its simultaneous neurotransmitter release in the other part of the tissue. The technique allows the simultaneous or different electrical stimulation of the two tissue areas. Furthermore, various compounds can perfundate through the separated, but anatomically connected tissue areas (even independently of each other, but also simultaneously).

The development of the system and related experiments were conducted in the research laboratory of Servier / Egis (L. Hársing Zs. Jurányi et. Al.). The application possibilities and advantages of the system are presented in the results of two experiments:

1. Schizophrenia model

In the research of neurochemical background of schizophrenia, it is a well-known practice to remove the striatum from the brain and place it into a one-volume perfusion chamber, to measure [3H]-dopamine release in the presence of various compounds. Two nerves make connection in the striatum, where one originates from the cerebral cortex and the other from the s. nigra. The death of the latter one develops Parkinson's disease, while the reduced operation activity of the former one plays a role in the development of schizophrenia. According to literature data, neurochemical experiments performed on the striatal slices in a one-volume perfusion chamber often show sharply contrasting results. During the preparation of striatal slices the cutting of the mentioned nerve pathways is inevitable. The resulting preparation contains both nerve endings, but does not include the base nerve cells and the intact pathways themselves. To achieve the appropriate examination of such brain slices are necessary, which include the cerebral cortex, the intact pathways and the striatum itself (where the track ends).

During the experiment the cortex is located in one half of the chambers, while the striatum is located in the other half. The tissue relationship remains between the two tissues (corpus callosum), but the communication between the two fluid compartments is inhibited. A plexiglass spacer plate can be fitted between the two vessels of the open organ chamber. The inserted plexiglass spacer plate divides the chamber into two compartments, each with 3 ml of volume. The brain slice can be positioned in fluid space in such a way that the cortical part and the striatal parts are separated in the line of the corpus callosum. Thus the cortical part will be in on compartment and the striatal part will be in the other compartment. The separating wall and the inserted tissue are surrounded by a high purity silicon grease, so no liquid flow is possible between the two compartments. The balanced flow of the solution is provided by a peristaltic pump.

The electrical stimulation of tissue is provided by the platinum electrodes built into the bottom of the compartments in the form of space stimulation (the tissue is situated between the two electrodes). Ag / AgCl electrodes built into the sidewalls can help to detect depolarization during nerve activity and the potential differences between the two chamber spaces.
The electrical stimulation of the cortex depolarizes the dopaminergix axon terminals in the cortical part of the slice, they release their [3H] dopamine content and it appears in the affluent. Simultaneously, as a result of the electrical stimulation, the stimulus of the corticostrial glutamate pathway is formed. The endogen glutamate released from the pathways ending in the striatum stimulates the [3H] dopamine release in the nigrostrial dopaminergic endings. Since the cortical part of the slice is located between the stimulating electrodes, the electrical stimulation has a direct effect on the tissue. In contrast to that, the [3H] dopamine release observed in the striatum occurs only subsidiarily as an effect of cortical stimulation.

With this combined wedge-release methodology it is possible to test the neurotransmitter release occurring simultaneously or but spatially separated in origination and termination areas, and neuronal depolarization during nerve activity.

2. Ischemia, atherosclerosis model

In case of circulatory failure (heart failure, cardiac ishcaemia, myocardial infarction) or the narrowing of the arteries of the central nervous system (atherosclerosis) a temporary or permanent hypoxia, ischemia occurs in the brain tissue. The advantage of the experimental system is that due to the dual-chamber design the affect of hypoxia in a given area of the nervous system can be modeled to another area unaffected by hypoxia. Hypoxia, ischaemia-like state can be created in one side of the right brain slice, cut from the nervous system (in one half of the chamber), while in the other side the perfusion of the normoxic, glucosous physiological solution can still be maintained. Meanwhile, electrical and/or chemical stimulation may be used (even on both sides) and pathological conditions envoked neurotransmitter and potential changes can be studied at the same.

The figure presents an easily-measurable potential change in the cortex, which was evoked by experimental ischemia in one side of a complex brain slices (N2 / CO2 gas saturated glucose-free Krebs solution perfundation). Since the two Ag / AgCl electrodes measure the potential difference between the cortex and the striatum, the potential change actually occurs between the cortex and the striatum.
The advantages of the system:

1. One of the outstanding benefits of the open perfusion system as opposed to closed systems, is that the test compounds can be injected directly next to the tissue in the chamber.

2. The grease-gap methodology with an appropriate precision perfusion chamber is suitable for studying radioactive neurotransmitter release in complex brain slices.

3. The two sides of the dissection can be perfused independently, which allows simultaneous perfusion of different pharmaceuticals in both sides of the tissue.

4. The two sides of the dissection can be electrically stimulated independently from each other, which allows the examination of the operation and impact of projection pathways, originating from one half of the tissue slice and ending in the other half. If necessary, both sides of the slice can be stimulated (either simultaneously or alternately).

5. The integrated Ag / AgCl electrodes aid to study the potential changes in the tissue slice, which supply information about the impact that the neuronal operation of the compound develops.
# Table of contents

**NEU-01 four channel in-vitro superfusion (release) systems**

- General Information  
  Page 2

- Components  
  Pages 3-4

- How can the system help?  
  Pages 5-6

**NEU-02 in-vitro superfusion system (cortical wedge technique)**

- General Information  
  Page 7

- Components  
  Pages 8-10

- How can the system help?  
  Pages 11-13

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