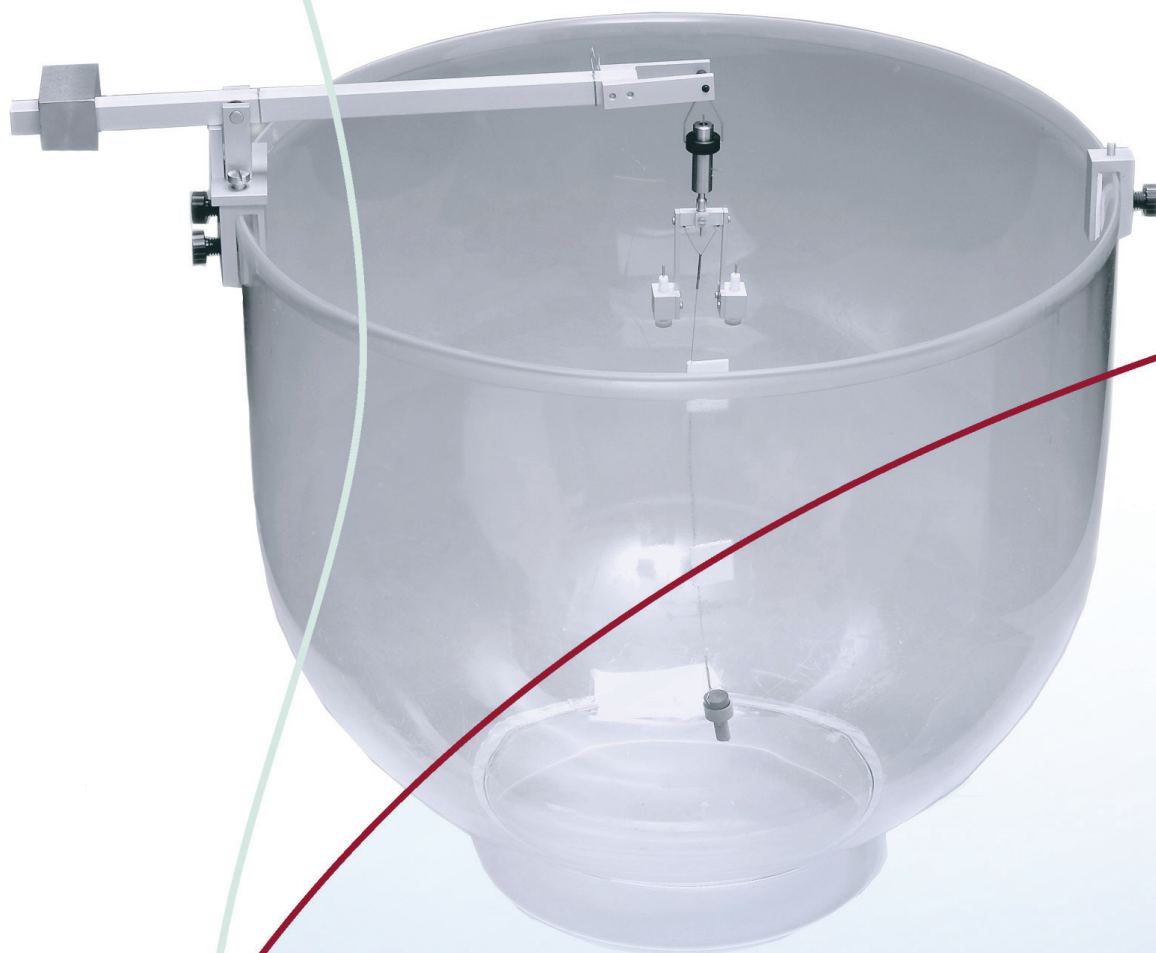


CMA 120

**System For Freely Moving Animals**



 **cma**  
a division of **Harvard Bioscience, Inc.**

# **CMA 120 SYSTEM FOR FREELY MOVING ANIMAL, USER'S MANUAL**

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## 1. INTRODUCTION

The CMA 120 System for Freely Moving Animals (see Figure 1) is designed for microdialysis in Conscious, Freely Moving animals. The system allows parallel studies of animal behaviour and sampling of biochemistry.

The CMA 120 System for Freely Moving Animals is equipped with a Dual Channel Swivel making the system very flexible and permitting many application possibilities, for example:

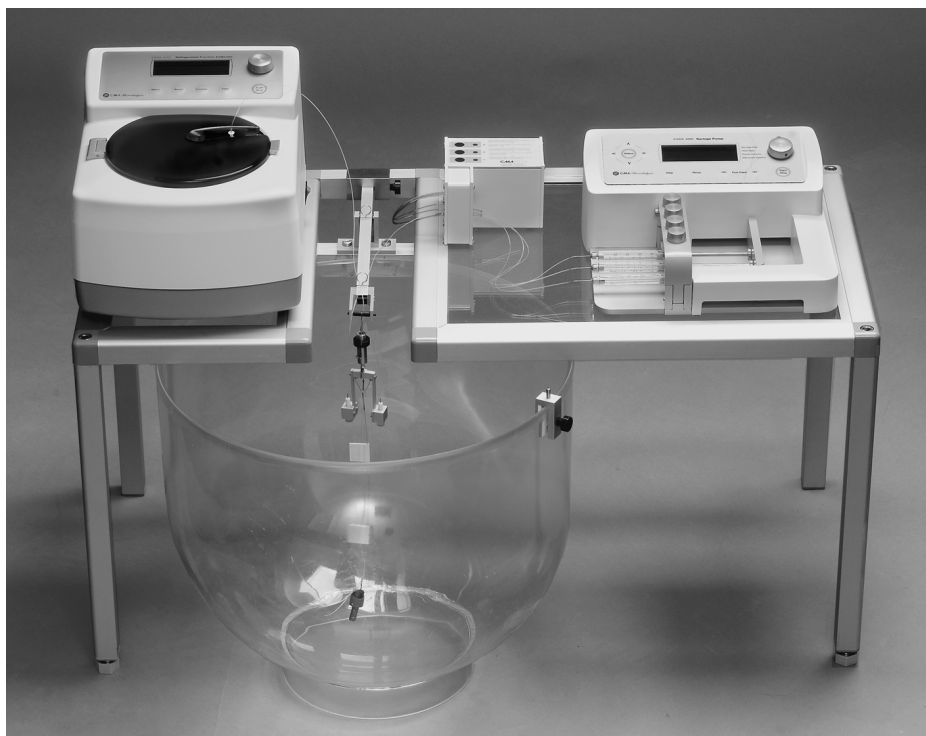
- Manual collection using one Microdialysis Probe.
- Manual collection using two Microdialysis Probes.
- Automatic collection using one Microdialysis Probe.
- Microdialysis combined with local injection.

The CMA 120 System for Freely Moving Animals will function with different kinds of Microdialysis Probes such as the CMA 11 and the CMA 12 Microdialysis Probe. A special Guide Cannula has been developed for each type of the Microdialysis Probe. The Guide Cannula is implanted stereotactically under anaesthesia and the animal is allowed to recover from the surgery for several days. The Microdialysis Probe is inserted through the Guide Cannula into the brain of the animal while it is conscious without any more distress to the animal than the handling. The insertion in itself does not cause any pain.

There are many advantages in using the Guide Cannula:

- The desired insertion zone for the probe remains totally undamaged.
- The animal can recover fully from the surgery.
- The Microdialysis Probe can be reused.

Depending upon the type of investigation, an experiment can be performed directly after the insertion of the probe or the probe may be left in the brain for a period of time before the experiment.



**Figure 1.** Example of an application of the CMA 120 System for Freely Moving Animals.

## **2. UNPACKING & ASSEMBLY**

The CMA 120 System for Freely Moving Animals is delivered in a specially designed box to protect the equipment against damage during transportation. The reusable carton provides excellent protection if it should be necessary to transport the equipment or if it is to be stored for a long period of time.

### **2.1 PACKING LIST**

- Bowl-Cage
- Counter Balancing Arm
- Dual Channel Swivel Device
- Collar
- Clamp for In Vitro Holder
- Tubing for connecting syringe to swivel
- Sample Vials
- User's Manual

After unpacking the box, check the contents against the above packing list to ensure that the shipment is complete. Impact all items for damage or missing parts should be reported immediately to CMA Microdialysis AB or your local supplier.

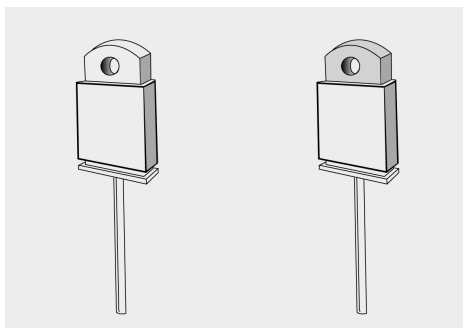
### 3. DESCRIPTION

#### 3.1 CMA 11 and the CMA 12 Guide Cannulae

The Guide Cannulae for the Microdialysis Probes have been developed for chronic implantation in the brain of rats and larger animals. The Microdialysis Probes and the Guide Cannulae are factory-made with such precision that the different guide and probes fit each other without any individual calibration.

A dummy is fitted in the Guide Cannula to stop the cannula from becoming blocked. The dummy is removed before inserting the Microdialysis Probe.

The Guide Cannula for the CMA 11 and CMA 12 Microdialysis Probes are cemented directly to the skull. The Guide Cannula is made of plastic and may easily be cut to the desired length. See figure 3.



**Figure 3.** Guide Cannulae for the CMA 11 and the CMA 12 Microdialysis Probe.

When introducing chronic implants into an animal, it is important to maintain semi-sterile conditions. Before use, the Guide Cannula can be sterilized with 70% alcohol and rinsed in sterile saline. It is also possible to sterilize the probe in 70% alcohol followed by a rinse with sterile saline. During perfusion, the membrane protects the brain from contact with a non-sterile perfusion liquid.

If needed the cannula of the Guide Cannula can be cut to desired length. The steel cannula and the steel dummy of the Guide Cannula may be cut in a lathe. The plastic cannula of the CMA 11 and the CMA 12 Guide Cannulae may be cut with a scalpel and the steel dummy in lathe. Deburring may be necessary before implantation.

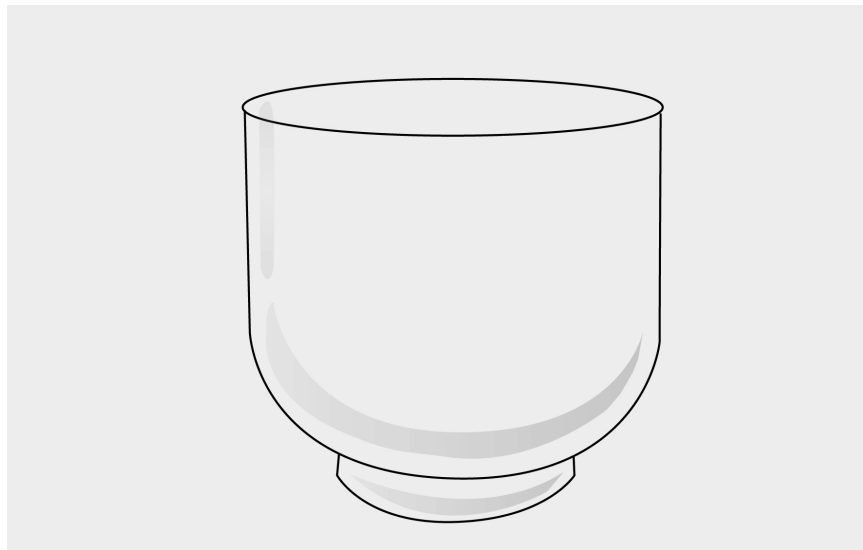
### 3.2 BOWL-CAGE

The Bowl-Cage is designed to prevent the animal from knocking the implant on the wall of the cage, see figure 4.

If desired, the Bowl-Cage can easily be painted a dark colour with cellulose paint.

It is possible to connect a water supply and food hopper to the Bowl-Cage if the animal is to remain in the cage for a long time.

Bedding material covering the bottom of the Bowl-Cage is recommended during an experiment.



**Figure 4.** Bowl-Cage.

### 3.3 COUNTER BALANCING ARM

The Counter Balancing Arm (see figure 5) keep the probe tubing away from the animal. The swivel is located on one side, a weight on the other. The weight should be adjusted so that the arm easily follows the animal's movements.

The Counter Balancing Arm can either be fixed to the wall of the Bowl-Cage or to an Instrument Table, see Ordering Information. The Counter Balancing arm is supplied with two clamps which are used to fix the arm to the Bowl-Cage. By unscrewing and removing the clamps, the Counter Balancing Arm can easily be fixed to the Instrument Table.

A steel wire from a collar fitted around the animal's neck to the swivel helps to move the arm up and down and turn the swivel. The animal can then move freely around in the Bowl-Cage without coming into contact with the tubing.

**Figure 5.** Counter Balancing Arm with tubing connections.

### **3.4 DUAL CHANNEL SWIVEL DEVICE**

A1: Inlet of the Centre Channel  
A2: Outlet of the Centre Channel  
B1: Inlet of the Side Channel  
B2: Outlet of the Side Channel

**Figure 6.** Dual Channel Swivel Device

The CMA 120 System for Freely Moving Animals is equipped with a Dual Channel Swivel Device. This consists of a Swivel Gimbal, a Dual Channel Swivel and two Vial Holders, see figure 6.



## Swivel Gimbal

The Swivel Gimbal is fixed to the Counter Balancing Arm in such a way that it is easily replaceable. See figure 5.

## Dual Channel Swivel

A Dual Channel Swivel with the CMA 120 System for Freely Moving Animals increases the application possibilities.

The Dual Channel Swivel is a freely turning swivel with two channels, one straight (Centre Channel) and one angled (Side Channel). The two channels have different internal volumes which is important to remember when connecting the tubing to the Dual Channel Swivel (see below).

The approximate internal volumes of the Centre and Side Channels are 1.4  $\mu\text{l}$  and 18  $\mu\text{l}$ , respectively.

The internal volumes can be exactly measured as follows:

- Fit a small microsyringe (25–50  $\mu\text{l}$ ) filled with water in the CMA 400 Syringe Pump.
- Connect the inlet of the channel to the microsyringe using FEP-tubing and Tubing Adapters.
- Run the pump at a low flow rate while observing the water inside the tubing with a stereo microscope. When the water just reaches the inlet side of the channel, stop the pump.
- Reset the delivered volume on the pump display and restart the pump. Water will now enter the channel.
- Using a stereo microscope, observe the outlet of the channel and as soon as water comes out, stop the pump.
- Read the delivered volume on the pump display.
- Repeat at least three times and calculate the mean internal volume of the channel.

**NOTE: The Dual Channel Swivel has undergone a special cleaning procedure before delivery. This prevents degradation of the substances per fused through the channels of the swivel.**

The Dual Channel Swivel can be used as a Single Channel Swivel by just using one of the channels.

## Vial Holders

The Dual channel Swivel is equipped with two Vial Holders which are designed so that the dead volume in the system is as small as possible when collecting samples. In addition, the animal is only minimally stressed when changing fractions.

The outlet from the probe carries liquid to the bottom of the inverted vial fixed to the rotating stand of the swivel. Thus the vial rotates with the swivel.

The vial is fitted inverted on the vial holder on the swivel. The outlet tubing of the probe is pushed from underneath into the vial until it reaches the bottom. The plastic sleeve is then pulled down to secure the tubing, see figure 7.

**NOTE:** The tubing must reach the bottom of the vial to make sure that the perfusion fluid stays in the vial.

**Figure 7.** Secure the outlet tubing in the Vial Holder by pulling down the plastic sleeve.

If a large volume ( $>50\mu\text{l}$ ) is to be collected, the liquid may fall down from the vial bottom. In this case, unscrew the Vial Stand and turn it through  $180^\circ$ . The outlet tubing will need to be extended by about 30-50 mm. The samples will now be collected down into the vial instead of up into the vial, see figure 8.

**Figure 8.** Inverted Vial Holder.

A steel wire extending from a collar on the animal turns the swivel. The tubing is taped to the two flags on the wire for support.

For tubing connections, see Connecting the Dual Channel Swivel.

### **3.5 CMA 11 AND CMA 12 MICRODIALYSIS PROBE**

The design CMA 11 and CMA 12 Microdialysis Probe is shown in figure 10.

The inner cannula of the CMA 11 Microdialysis Probe consists of fused silica. The steel shaft has an outer diameter of 0.32 mm and a length of 14 mm. The membrane outer diameter is 0.24 mm.

**NOTE: Due the fragility of the CMA 11 Microdialysis Probe, it must be handled with special care.**

The inner cannula of the CMA 12 Microdialysis Probe consists of steel except for the outlet that consists of fused silica. The steel shaft has an outer diameter of 0,64 mm and a length of 14 mm. The membrane outer diameter is 0,5 mm.

**Figure 10.** CMA 11 and CMA 12 Microdialysis Probes.

The CMA 11 and CMA 12 Microdialysis Probes are delivered without tubing. That means that the inlet and the outlet cannula of the probe has be attached to a appropriate length of tubing. Tubing Adapter and FEP-Tubing are recommended for all connections.

Perfusion liquid enters at the top of the probe and flows down the inner cannula tube and leaves at the lower end of the tube. The liquid then flows upwards inside the dialysis membrane. It then enters an outlet silica tube and leaves the probe through the steel outlet tube.

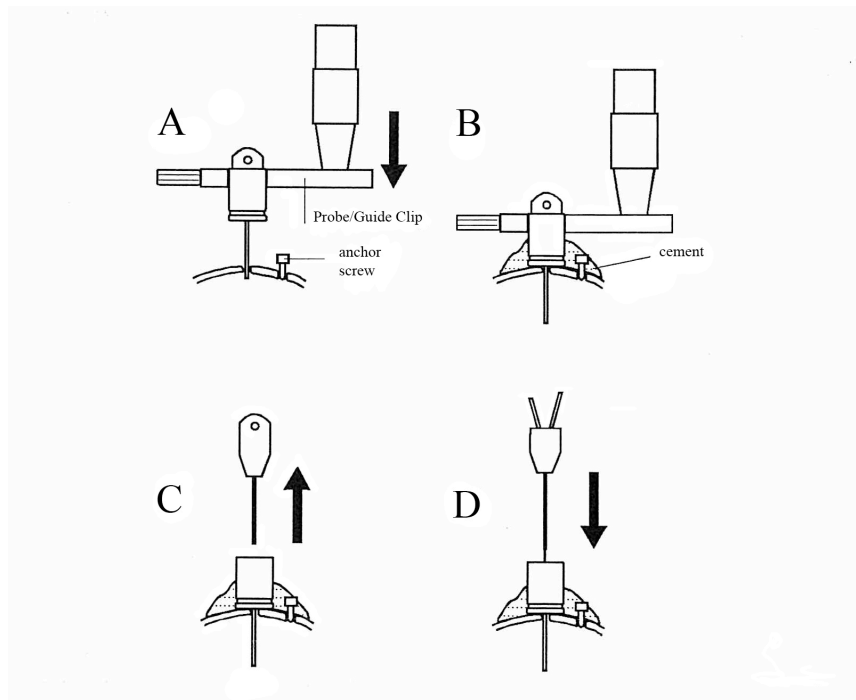
The material used in the CMA 11 and CMA 12 Microdialysis Probes are specially treated to reduce degradation of substances in the perfusate. In most cases no antioxidants or similar sub-stances need to be added to the perfusate.

The dialysis membrane is made of a specially developed material with very good diffusion characteristics and excellent biocompatibility. The molecular weight cut-off is available in 6000-100,000 Daltons.

## 4. INTRODUCTION FOR USE

### 4.1 IMPLANTATION OF THA GUIDE CANNULA

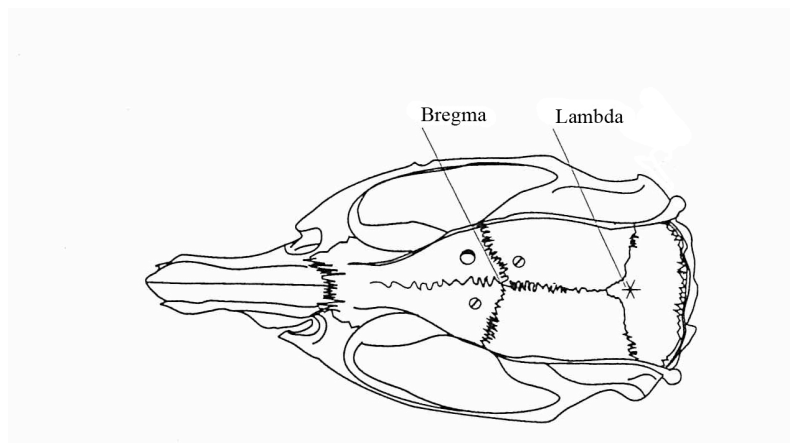
1. Mount the guide cannula in the CMA 11 + 12 Clip and fix the clip to the Connecting Rod. See figure 11.
2. Attach the assembly to the micromanipulator of the stereotaxic frame.



**Figure 11.** Implantation of the CMA 11 or CMA 12 Guide Cannula (from side).

A: The Guide Cannula fitted in the Probe/Guide Clip is lowered into the brain.  
B: The Guide Cannula is cemented to the bone and the anchor screws.  
C,D: The dummy is removed before inserting the Microdialysis Probe.

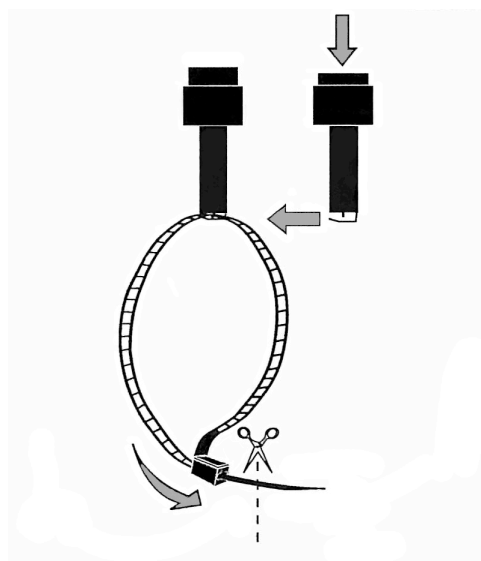
3. Anaesthetize the animal and place it in a Stereotaxic Instrument. Shave the head of the animal and make a sagittal midline incision through the skin to expose the coronal and transverse suture.
4. Drill a hole where the Guide Cannula is to be implanted. It is preferable to use a Trephine Drill Bit which cuts clean holes without damaging the brain. Determine the position of the hole using Bregma coordinates, pointing with the guide. Drill 2-3 holes for the anchor screws positioned in different bones of the skull using an anchor screw drill Bit. Screw the anchor screws into the skull just so that they go through the bone. See figure 12.
5. Bring the cannula of the Guide into contact with the dura of the brain to determine the zero value for the vertical coordinate. Open the dura carefully with the tip of a hypodermic needle. Lower the cannula of the guide slowly to the desired depth in the brain.



**Figure 12.** Close-up of the skull with a Trephine-drilled hole and two anchor screws placed in different bone plates.

**NOTE:** To calculate the implantation depth for the Guide Cannula, subtract the probe's membrane length from the desired depth for the probe.

6. Cement around the guide and the anchor screws, see figure 11.  
Use a type of cement that cures without producing heat which can damage the skull and the underlying cortex.  
Suture the skin above the cement.  
Fix the special collar around the neck of the animal, see figure 13.



**Figure 13.** Collar with Clip

7. Before inserting the Microdialysis Probe, allow the animal to recover until it has the same weight as before surgery (3-4 days), or until the wounds are healed.

**NOTE:** During the recovery period of the animal, keep it in a cage where the implant cannot get caught in any metal grid.

## **4.2 CONNECTION OF THE DUAL CHANNEL SWIVEL**

The use of FEP-tubing with Tubing Adapters is recommended for all connections.

The CMA 110 Liquid Switch or CMA 111 Syringe Selector can be connected between the CMA 400 Syringe Pump and the Dual Channel Swivel.

### **Manual collection using one Microdialysis Probe.**

#### **Connect tubing as follows:**

- From the CMA 400 Syringe Pump to the inlet (A1) of the Centre Channel.
- From the outlet (A2) of the Centre Channel to the inlet tubing of the Microdialysis Probe.
- From the outlet tubing of the Microdialysis Probe to one of the Vial Stands.
- See figure 14.

**Figure 14.** Manual collection using one Microdialysis Probe.

## **Manual collection using two Microdialysis Probe**

### **Connect tubing as follows:**

#### **Probe 1:**

- From the CMA 400 Syringe Pump to the inlet (A1) of the Centre Channel.
- From the outlet (A2) of the Centre Channel to one of the Microdialysis Probes.
- From the outlet tubing of the Microdialysis Probe to one of the Vial Stands.

#### **Probe 2:**

- From the CMA 400 Syringe Pump to the inlet (B1) of the Side Channel.
- From the outlet (B1) of the Side Channel to the inlet tubing of the other Microdialysis Probe.
- From the outlet tubing of the Microdialysis Probe to the other Vial Stand.
- See figure 15.

**NOTE:** In this application, it must be decided which Microdialysis Probe is the better to have connected to the Centre Channel which has a significantly lower internal volume.

**Figure 15.** Manual Collection using two Microdialysis Probes.



### **Automatic collection using one Microdialysis Probe**

By using the CMA 142 Microfraction Collector or the CMA 470 Refrigerated Fraction Collector, it is possible to collect samples automatically.

#### **Connect tubing as follows:**

- From the CMA 400 Syringe Pump to the inlet (B1) of the Side Channel.
- From the outlet (B2) of the Side Channel to the inlet tubing of the Microdialysis Probe.
- From the outlet tubing of the Microdialysis Probe to the outlet (A2) of the Centre Channel.
- From the inlet (A1) of the Centre Channel to the CMA 142 Microfraction Collector or the CMA 470 Refrigerated Fraction Collector.
- See figure 16.

**NOTE:** If the outlet of the Microdialysis Probe is connected to the Centre Channel (with the lower internal volume) instead of to the Side Channel, the sample will reach the sampling vial by the faster route.

**Figure 16.** Automatic collection using one Microdialysis Probe.

## **Microdialysis combined with local injection and manual collection**

### **Connect tubing as follows:**

#### **Probe:**

- From the CMA 400 Syringe Pump to the inlet (A1) of the Centre Channel.
- From the outlet (A2) of the Centre Channel to the inlet tubing of the Microdialysis Probe
- From the outlet tubing of the Microdialysis Probe to one of the Vial Stands.

#### **Injection:**

- From the CMA 400 Syringe Pump to the inlet (B1) of the Side Channel.
- From the outlet (B2) of the Side Channel to the injection catheter implanted in the animal.
- See figure 17.

**NOTE:** It is important to choose the correct channel for the experiment. For example, if a valuable drug is being injected – use the Centre Channel for the injection. If the perfusion medium is to be changed during the experiment – use the Centre Channel for the Microdialysis Probe.

**Figure 17.** Microdialysis combined with local injection.

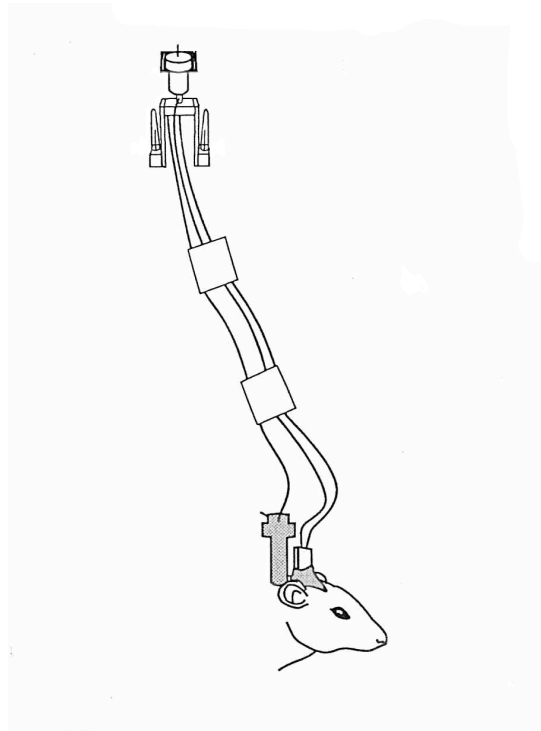
### 4.3 INSERTION OF THE MICRODIALYSIS PROBE

1. Connect the tubing to the swivel as required, see 4.2.  
If necessary, connect the CMA 110 Liquid Switch or the CMA 111 Syringe Selector between the CMA 400 Syringe pump and the Dual Channel Swivel. This is not necessary unless the experiment involves changing of the perfusion fluid.  
Run the pump to make sure that liquid leaves the outlet of the swivel.  
Make sure that the Counter Balancing Arm is locked using the “stopper”, see figure 5.
2. Connect the Microdialysis Probe to the Dual Channel Swivel and prepare it by following the procedure described in the manual for the Microdialysis Probe.  
Place the Microdialysis Probe in the CMA 130 In Vitro Stand with an Eppendorf vial filled with perfusion liquid. The Eppendorf vial can also be fixed to the edge of the Bowl-Cage with a Vial Holder. Use the Probe/Guide Clip and Vial Holder from the CMA 130 In Vitro Stand for securing the probe, see Ordering Information.  
Connect tubing to the probe and flush the probe with perfusion fluid. See Probe manual.
3. While holding the animal, attach the steel wire to the collar around the neck of the animal using the clip, see figure 13 and 19.  
The animal can be allowed to become used to the collar during the recovery period.
4. Remove the dummy from the implanted Guide Cannula and insert the Microdialysis Probe while holding the animal firmly against your body. This requires some practice as the probe can easily be damaged if the animal moves its head during insertion, see figures 18.  
The CMA 11 and the CMA 12 Microdialysis Probe are automatically secured when gently pressing it down into the Guide Cannula.



**Figure 18.** Holding the animal while securing the Microdialysis Probe

5. Release the “stopper” on the Counter Balancing arm, see figure 5. Place the animal in the Bowl-Cage. Make sure that the animal has enough free tubing to move its head in all directions, before taping the inlet and outlet tubing to the “flag” on the steel wire, see figure 19.



**Figure 19.** Steel wire with flags and clip.

6. The system is now ready for use. The microdialysis experiment can be performed in the same way as with an anaesthetized animal.

**NOTE:** Be sure to take into account the dead volume of the system when changing fractions and giving drugs!

#### **4.4 INSERT OF THE MICRODIALYSIS PROBE INTO THE GUIDE CANNULA WHEN THE PROBE IS NOT TO BE PERFUSED IMMEDIATELY**

1. Implant the Guide Cannula as described above and allow the animal to recover.
2. Prepare and perfuse the Microdialysis Probe according to the manual. Insert the probe through the Guide Cannula and secure it while continuously perfusing the probe.
3. Cut the inlet (text to be added) and outlet tubing of the probe to approximately 5 cm length. Connect the two tubing together by the use of a Tubing Adapter.
4. Return the animal to its cage. Use a cage where the implant and the tubing cannot get caught in any metal grid such as the food hopper.
5. When the experiment is to begin, cut the distal ends of the inlet and outlet tubing of the probe to remove the Tubing Adapter and make sure that liquid fills the tubes completely. Attach appropriate lengths of FEP-tubing to the swivel and to the vial collecting stand. Perfuse the swivel and its channel to make sure that there is no air trapped in the system. It is particularly important that the Tubing Adapters are filled with perfusion liquid to prevent air bubbles entering the probe. Connect the animal to the swivel. (Two persons may be required, one to hold the animal, and one to connect the tubing.) The experiment is then performed as described above.

#### **4.5 EXAMPLE OF DIFFERENT SET-UPS FOR THE CMA 120 SYSTEM FOR FREELY MOVING ANIMALS.**

##### **Example 1:**

- One or two Microdialysis Probes implanted in the animal.
- Manual sample collection.

##### **Example 2:**

- One Microdialysis Probe implanted in the animal.
- Automatic sample collection using the CMA 142 Microfraction Collector or the CMA 470 Refrigerated Fraction Collector.

##### **Example 3:**

- One Microdialysis Probe implanted in the animal.
- Changing perfusion fluid using the CMA 111 Syringe Selector.
- Automatic sample collection using the CMA 142 Microfraction Collector or the CMA 470 Refrigerated Fraction Collector.

##### **Example 4:**

- One Microdialysis Probe and one injection catheter implanted in the animal.
- Changing perfusion fluid for the Microdialysis Probe using the CMA 111 Syringe Selector.
- Automatic sample collection using the CMA 142 Microfraction Collector or CMA 470 Refrigerated Fraction Collector.
- Giving a systemic injection to the animal by using a second CMA 400 or 402 Syringe Pump.

## **5. MAINTENANCE**

### **5.1 CLEANING THE BOWL-CAGE**

The cage can be cleaned with soap and water, and alcohol if required.

### **5.2 CLEANING THE DUAL CHANNEL SWIVEL**

It is essential that the channels of the swivel are cleaned after use. If this is not done, salt crystals from the perfusion fluid may form, which can block and seriously damage the channels.

**NOTE:** Always clean the channels of the swivel after use by perfusing with distilled water.

### **5.3 CLEANING A CRYSTAL-BLOCKED DUAL CHANNEL SWIVEL**

If salt crystals have blocked the channels of the swivel, proceed as follows:

- Flush with distilled water  
If that does not work:
- Flush with hot distilled water.  
If that does not work:
- Contact CMA Microdialysis AB or your local supplier for advice.

### **5.4 STORAGE**

If the CMA 120 System for Freely Moving Animals is not to be used for a significant length of time:

- clean all part of system.
- store system in shipping carton in safe place.

The above suggestions will help keep your CMA 120 System for Freely Moving Animals running smoothly and in good condition.

For advice, service or technical assistance, contact your local supplier or CMA Microdialysis AB.

## **6. TECHNICAL DATA**

### **6.1 CMA 11 Guide Cannula**

Overall length: 25 mm  
Outer dimensions (rubber section): 2.5 x 7 x 10 mm  
Outer diameter (tubing): 0.6 – 0.7 mm  
Length (tubing from rubber): 10 mm  
Material: Tubing – Polyurethane  
Rubber section – Santoprene

### **6.2 CMA 12 Guide Cannula**

Overall length: 25 mm  
Outer dimensions (rubber section): 2.5 x 7 x 10 mm  
Outer diameter (tubing): 0.9 mm  
Length (tubing from rubber): 10 mm  
Material: Tubing – Polyurethane  
Rubber section – Santoprene

### **6.3 BOWL-CAGE**

Height: 360 mm  
Diameter: 400 mm  
Material: Perspex

### **6.4 DUAL CHANNEL SWIVEL**

Housing dimensions: 9.5 x 33 mm  
Internal volumes: Centre Channel = 1.4 µl  
Side Channel = 18 µl

### **6.5 SERVICE**

CMA and CMA distributors have skilled service staff to solve your technical problems if an equipment-oriented problem should arise. For further details, call/email/fax first your local CMA distributor and secondly direct to CMA.

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## 7. ORDERING INFORMATION

Name	Ref No.
<b>CMA 120 System for Freely-Moving Animals</b>	8309049
<b>Accessories</b>	
Instrument table	8309046
CMA 120 System without Bowl	8409029
CMA 120 Balance arm	8309032
CMA 120 Plastic bowl	8309031
CMA 120 Swivel assembly	8309048
CMA 120 Swivel assembly w/o swivel	2409090
CMA 120 Wire set	2409051
Gimbal for CMA Swivel	8002714
Swivel, Dual Channel	8409047
Swivel, Dual Channel, MD mouse	8001346
Plastic Collar, 100/pkg	7431059
CMA 11 Guide Cannula 3/pkg	8309012
CMA 11 Guide Cannula 30/pkg	8309015
CMA 12 Guide Cannula 3/pkg	8309014
CMA 12 Guide Cannula 30/pkg	8309016
Anchor screws 100/pkg	7431021
Tubing Adapter 10/pkg	3409500
FEP-Tubing 1 x 1m	3409501
FEP-Tubing 10 x 1m	8409501
Trephine Drill Bits 3/pkg	3409500
Anchor Screw Drills 3/pkg	8003264
Plastic Vial 1000/pkg	7431033
Caps Plastic Resealing, 1000/pkg	7431102
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Kopf Adapter	8309005

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