## Melatonin Window of Treatment Study: NIRS Protocol

## **Protocol**

In this study melatonin (or ethanol) is administered intravenously for 6h, starting 10min, 2h or 4h post-HI. This is repeated 24h later (infusion starting at 24:10, 26 or 28).

In order to monitor the effects of the melatonin infusion, we take NIRS measurements throughout the 6h, from 5min before the start until 10min after the end of each infusion. Both, initial and repeated infusions are monitored this way.

If the melatonin infusion starts 10min after the insult the 31p- and melatoninmeasurements overlap. In this case we leave the 31p-measurement running until the end of the insufion (ideal) or, if the pig must be moved, we stop and save the 31p-measurement just before the transport and start the melatoninmeasurement once the pig is settled in the recovery room. In either case a note on the exact procedure must be taken down in the lab book.

## How to use the NIRS System

**Start Data Acquisition:** Make sure that light and heating are turned off and the pig's head is covered with black cloth.

At the NIRS-System PC, click the red button once. Then press the 'NIRS Gate' button next to the main PC and a blue light should come on. In the NIRS lab book, write down the time displayed on the main PC.

Approximately 30s after the NIRS data acquisition was started the spectra and concentrations should refresh every 10s.

**During Acquisition:** We obtain the best NIRS data when the pig is moved as little as possible. In case emergency treatment is necessary, the lights are turned on, the head is uncovered etc. please make a note in the log book including the time and a short description of what happened. Only if the optodes have to be repositioned or removed the data acquisition needs to be iterrupted.

Stop Data Acquisition and Save: In order to stop the data aquisition click the red button again. Then, save the file ('File'--'Save') naming it in the format 'mel\_ddMonthYYYY.tdf' (Example: 'mel\_14July2014.tdf'). Finally, press the 'NIRS Gate' button again and the blue light should go out.

The main PC should be left running. The NIRS-System PC can be shut down  $after\ closing\ Taragona.$