Supplementary Information

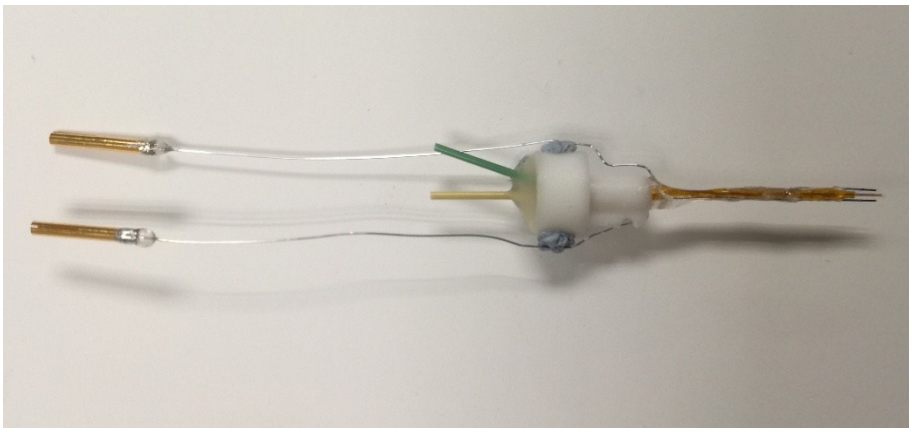
In vivo characterisation of a catalase-based biosensor for real-time electrochemical monitoring of brain hydrogen peroxide in freely-moving animals.

Saidhbhe L. O'Riordan[†*] and John P. Lowry[†]

[a] *†Neurochemistry Research Unit, BioAnalytics Laboratory, Department of Chemistry, Maynooth University, Maynooth, Co. Kildare, Ireland.*
*Corresponding author
E-mail: Saidhbhe.oriordan@nuim.ie
Tel: +353 1 708 6571.

Surgical protocol (combined MD probe/dual H_2O_2 sensor implantation)

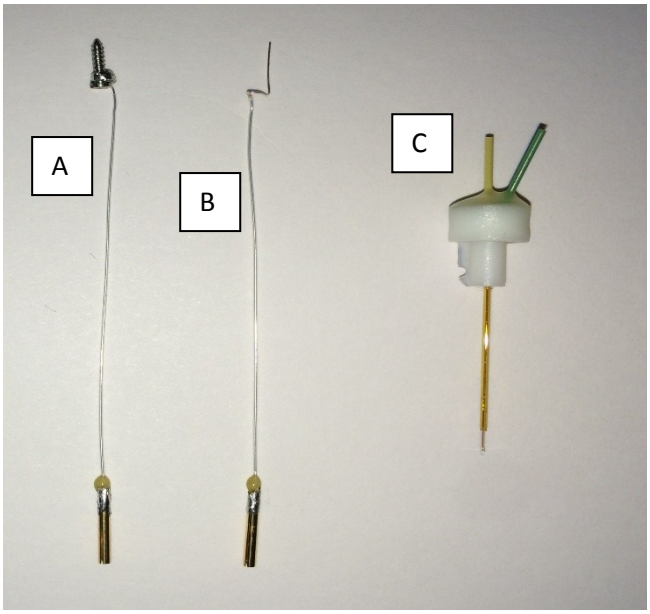
As briefly discussed in the main text, each working electrode (i.e. *PEROX* and *CONTROL*) was positioned accurately, with the use of a microscope, at either side of an MD probe. Care was taken to ensure that the orientation of both sensor types were mirrored exactly on opposite sides of the MD probe. Each electrode type (i.e. *PEROX* and *CONTROL*) was secured in place, flush to the cannula, using epoxy resin to ensure stability of each working electrode for the purpose of surgical implantation. The active surface of each electrode type (i.e. *PEROX* and *CONTROL*) was retracted approximately 1mm from the point of implantation and terminal aspect of the MD probe membrane (2 mm length). The ventral extremity of each working electrode design (*PEROX*/*CONTROL*), in relation to the implant site, was precisely matched on both sides of the MD probe to ensure equal diffusion of each locally applied substance to the active surface of each working electrode. The image below (Supplementary image 1) is a typical example of the MD probe/dual H_2O_2 sensor construct orientation.



Supplementary image 1: MD probe/dual H_2O_2 sensor construct.

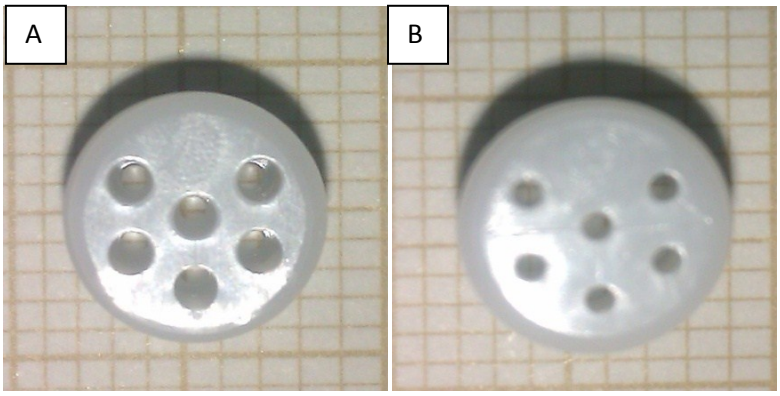
Supplementary image 2 demonstrates the reference and auxiliary electrode constructed in-house and utilised for *in vivo* experiments. Approximately 2 mm of Teflon® was removed from one end of the wire and soldered into a gold electrical contact (gold clip). The point of connection between the electrode and gold electrical contact was glued to provide the flexibility required to manipulate the sensors during surgery. At the opposite end of the wire *ca.* 3.5 mm of Teflon® was removed. To manufacture a reference electrode, the uncoated

wire was twisted in a manner, which would allow it to rest on the skull of the subject and to control the position of the wire ventrally as can be seen below. The auxiliary electrode was constructed in a similar manner to the reference electrode, however the exposed section of silver wire was soldered to a surgical screw. The auxiliary electrode was attached to a surgical screw to provide a larger surface area and to minimise the invasiveness of the surgical procedure by utilising a pre-existing implant. The reference electrode was placed in the cortex as discussed in the main text.



Supplementary image 2: Images of (A) auxiliary electrode and (B); reference electrode and (C) MD probe

The MD probe/dual H_2O_2 sensor construct was implanted in the striatum following the procedure described in the main text. The gold clips of the working electrodes, attached to the MD probe using a temporary support, were released and positioned for ease of placement within a plastic support as shown below in supplementary image 3. Once all electrodes were implanted and surgical screws were in place the electrodes were cemented at the point of insertion into the skull. The gold clips of each electrode, i.e. reference, auxiliary electrode and both working electrodes (*PEROX*/*CONTROL*) were then placed within this plastic support, which was a Teflon® pedestal as mentioned in the main text and each gold clip was cemented into the pedestal while ensuring that no cement entered the cavity of the gold clips.



Supplementary image 3: Images of Teflon®pedestal (A) Lower surface (B); Upper surface

The pedestal provided a physical method of connection between the gold clips of each electrode type and the potentiostat (4 channel Biostat, ACM instruments, UK), through a six-pinned flexible light-weight cable (Plastics-One, Roanoke, VA, USA) post-surgery. All electrodes were then manipulated to position them close to the surface of the skull and the MD probe, with the pedestal positioned centrally and vertically. The entire construct with the exception of the upper surface and sides of the pedestal were then cemented in place, eliminating the possibility of damage inflicted by the subject post-surgery and avoiding exposure to moisture. The surgical clamps were removed and the skin surrounding the cement was sutured. The post-surgical experimental and animal welfare monitoring protocol was carried out as discussed in extensive detail in the main text.