Q: What sort of RSNA electrodes are you using for recording for 6 weeks? Do you use SILGEL A & B? What is the success rate at 6 weeks post-surgery?

[J. Phillips]: We are using the Millar telemeter to record simultaneously renal sympathetic nerve activity (RSNA) and blood pressure (BP) in rats. We do use SILGEL in our experiments to provide insulation for the nerve and electrodes from surrounding connective tissue and to minimize possible noise in the recordings and ECG artefacts. In rats, we were able to report almost 70% success rate in our experiment out to two weeks but this dropped to 20% out to 6 weeks.

[F. McBryde]: Recordings of SNA are very difficult recordings to achieve and requires building up skills and expertise in the methodology. The success rate would depend a lot on how well the surgery is performed. Careful precise surgery is required for success given the fragility of the nerves; if they are stretched too much during surgery or the blood vessels supplying the nerve are damaged during surgery, the nerve would die and the recording will deteriorate.

Q: Have you recorded RSNA in SHR and SHRSP rats? If yes, for how long?

[F. McBryde]: I have recorded RSNA for 3 weeks in SHR rats (McBryde, Nat Comms, 2013) and LSNA for 2 weeks (Hart & McBryde, Hypertension, 2013).

Other authors in Japan have recorded both RSNA and LSNA for 4 weeks in SHR rats from 8-12 weeks of age (Mineyama, Yoshimoto, Shirai, Miki, FASEB J, 2013 27:1108.4), as well as in Zucker fatty rats (Shiwa, Yoshimoto, Miki, Okano. FASEB J, 2015 29:830.5), and Dahl-Salt-sensitive rats (Yoshimoto, Onishi, Mineyama, Shirai, Miki, FASEB J 2012, 26:875.10).

Q: Can you implant the ICP sensor into the cerebral ventricles to measure brain ventricular pressures?

[F. McBryde]: If placed into a ventricle the ICP sensor would give ventricular pressure. It is worth noting that several authors have found that intracranial pressure is consistent regardless of where within the cranium it is measured eg sub-dural vs intraventricular vs within brain tissue. An argument against an ICV placement would be that there would inevitably be damage to brain tissue on insertion of the catheter.
Q: How long does it take to implant the RSNA electrodes in a rat?

[S. Lau]: As Dr McBryde has pointed out, renal sympathetic nerve activity recordings are very difficult to perform, requires a lot of care and precision during surgery and is not a surgery than can be rushed. It can be expected that the surgical time to implant a BP and SNA telemeter for RSNA recordings would take over three hours for researchers who have not performed the surgery before but has some previous surgical experience in rats. Over time with experience, it can be expected a routine BP + RSNA surgery to take approximately two hours.

Q: How long can an O2 sensor last?

[F. McBryde]: A correctly placed oxygen sensor within the cranium should last for several weeks at least (Russell et al, J Neurosci Methods, 2012). This may differ in other organs where the sensor placement is less protected/stable, although even renal oxygen recordings have been shown to be stable for 3 weeks (Koeners et al, Methods Mol Biol, 2016; and Koeners et al, Am J Physiol, 2013).

We have found that the carbon electrodes themselves are stable enough to be stored for up to 12 months before attaching to the telemeter – but after any sort of handling (eg when attaching to the telemeter lead) they should always be recalibrated before implantation.

Q: What is the shape of the RSNA electrodes ..... 2.5 loops each?

[J. Phillips]: In our experiment, we used the recommended protocol suggested by telemetry research to shape our wires. Note that both wires are sitting within one outer big silicone tubing then each wire is further encased within another layer of silicone tubing. To fashion the electrodes, both wires are exteriorized to the side the outer silicone tubing at 2 adjacent points not more than 2 mm apart. The inner silicone tubing on each wire is then stripped off at the tip and the ends of both wires tied off with a surgical thread to prevent the monofilaments on each wire from going splay during handling. The wires are then shaped into hooks using fine forceps. Stocker and Muntzel have published an extensive review on how to obtain quality chronic SNA signals using recordings of not only RSNA but also lumbar and splanchnic SNA. They have suggested different methods to shape electrodes based on the nerve a researcher is interested in recording. I would greatly suggest referring to this paper (Stocker et al., 2013).


[S. Lau]: The RSNA electrodes on the telemeter are supplied straight, and can be carefully shaped for the particular methodology you intend to use for recording. There are multiple methods for recording
SNA and the shape of the electrodes will depend largely on the method you employ. In one method for recording RSNA, the electrodes have been shaped into a hook (see below).

Q: It looks like there is a time lag for change in tissue oxygen of approximately 5 minutes from time of exposure to hyperoxic hypercapnia, does that seem correct? Does that reflect what is happening in the tissue or is just an artifact of the technology?

[F. McBryde]: To induce hyperoxic hypercapnia, we place the animal and its cage in a box where we slowly replace the air inside with a 95% O₂ mixture. Therefore, I think that delay is reflective of the time it takes to replace the room air in the box and the tissue response.

Q: Is the RSNA signal clean enough to quantify burst frequency or bursts/100 beats? Why not quantify that way as opposed to microvolts?

[J. Phillips]: We did not report burst frequency in our experiments as in some experiments we were unable to calculate heart rate due to less pulsatile BP signal associated with the legacy Telemetry Research telemetry system which uses gel-filled fluid pressure catheters instead of the solid state pressure sensors. SNA has routinely been expressed as burst frequency (bursts/minute) or burst incidence (bursts/100 heart beats); however, this method of normalization still overlooks information regarding burst size. Some labs also avoid this method due to different resting heart rate between humans and rats. Muntzel group, however, calculated burst amplitude (μV) and frequency (Hz) in addition to percentage scores when reporting chronic elevation of lumbar SNA associated with obesity in female Wistar rats (Muntzel et al., 2012). So far the best method suggested to normalize SNA signal, albeit in rabbits, has come from Head’s lab (Burke et al., 2003). Head’s method, which normalizes SNA to the maximum sympathetic response induced by nasopharyngeal stimulation, allows for baseline comparison between animal groups and eliminates the effect of decay in SNA recorded over a prolonged period. Unfortunately, the method outlined does not appear to be transferable to rats because relative to nasopharyngeal stimulation in rabbits which predominantly recruits the trigeminal pathway, the stimulus primarily
employs the olfactory pathway, which has the added disadvantage of receiving some baroreceptor input (Salman, 2015).


Q: How long could you keep a stable SNA signal? Additionally, how long did you wait until getting a stable SNA baseline?

[J. Phillips]: We allowed the animal to recover from surgery for one week. After the recovery period, the baseline SNA recordings were generally stable. We have had success recording SNA for up to 6 weeks post-surgery.

Q: How did you calibrate SNA in microvolts? Did you pass a known current through the transmitters?

[S. Lau]: Millar Sympathetic Nerve Activity telemeters are supplied factory calibrated. Additional calibration is not required of the user.

Q: How is the pressure sensor calibrated long-term?

[S. Lau]: The solid state pressure sensor used in the Millar pressure telemeters are factory calibrated under tightly controlled conditions similar to what the telemeter would experience when implanted in an animal. Millar telemeters do not need to be re-calibrated periodically, or before each use.

Q: How do achieve SNA recording? Where and how do you attach the sensing element?

[S. Lau]: The methodology for recording SNA is dependent on the nerve of interest. For renal SNA, electrodes would be attached to the renal nerve. We recommend that you read the paper by Stocker & Muntzel, (Stocker, S. D., & Muntzel, M. S. (2013). http://doi.org/10.1152/ajpheart.00173.2013) for detailed methods pertaining to renal, lumbar or splanchnic nerve recordings.
Q: Did you have any complications after the implantation (of SNA implant)?

[J. Phillips]: Telemetry probe implantation is a major surgery as it involves multiple surgical incisions. Most of the complications we experienced, however, were observed in our sick model of CKD, the Lewis Polycystic Kidney (LPK) rat. The LPK body weight was a big challenge as the manufacture recommends implantation in rats that weigh more than 200 g and our rats are typically quite small for age. From our experience, rats that survive the first 3 days after surgery would generally have a very good recovery. We did not experience any surgical related infection complication after the surgery as rats were pretreated with antibiotic prior to the procedure. Weight loss after the surgery and dehydration was an issue and we aimed to maintain hydration using closely managed subcutaneous injection of fluids (e.g. saline and glucose). Providing pain relief (e.g. NSAIDs and/or opioids) and good quality readily digested food was also critical.

One major complication risk for implantation surgery especially in smaller animals is hind limbs stiffness due to BP sensor sitting in the aorta compromising blood flow to the legs. This can be observed during the procedure itself such that before recovery it was evident that there was leg stiffness and poor perfusion. Options to help if this is occurring are doing the RSNA surgery first then implanting the BP probe into the aorta (which is contrary to the manufacturer’s recommended protocol but means less time that the animal is immobile after the arterial probe insertion) or 2) implanting the BP probe inside the right femoral artery (this was easier with the previous Telemetry Research system where the BP probe was merely a fluid filled catheter that was easy to bend). The manufacturers do not recommended the femoral artery approach with the new Millar catheters as the sensor does not have enough flexibility and is more likely to kink.

Q: Did you see the same percentage increase in RSNA in the anesthetized prep as in the conscious rat using the Millar system?

[J. Phillips]: Although it might be inaccurate to compare both scenarios as different recording systems and conditions (anesthetized vs. conscious) were used, but the answer is yes. We observed approximately 50% higher RSNA resting levels in both conscious and anesthetized adult male LPK relative to control counterparts.

Q: How do you measure your brain O2 concentrations?

[F. McBryde]: Assuming that this question refers to calibration of the oxygen electrode before implantation? After attaching my three electrodes to the telemeter (the carbon “sensing” electrode, the auxiliary electrode and the ground electrode), I then calibrate these using different gas mixes. To get my “zero” signal I expose the electrodes to saline with 100% nitrogen bubbled through it (eg 0% oxygen), then to 10% oxygen and finally 21% oxygen. This works well for my brain oxygen measurements. If your organ of interest has a higher tissue oxygen content then you might like to use a higher oxygen concentration for calibration to ensure that you are covering the relevant range that you will be measuring in your tissue.
Once the telemeter has been calibrated before implantation, you must take great care not to disrupt the carbon electrode tip. Even touching it to a surgical drape or knocking it by mistake during sterilization or handling can affect the tip, and require recalibration.

Once the electrodes are inserted into the brain tissue, I then record the nA output of the telemeter on my acquisition software (Spike2 by CED instruments in my case), and apply the individual calibrations for that particular telemeter when I later analyze the data.

Q: In which brain area did you succeed the implantation of the Oxygen probe? Would it be possible to have measurement in a deep structure?

[F. McBryde]: I am currently making measurements in the cerebral cortex which is fairly shallow (2-3mm below dura). Previous studies from my colleagues have measured brainstem oxygen (Russell et al, J Neurosci Methods, 2012). To access this deep structure without damaging the delicate carbon electrode they cemented a guide cannula in place before inserting the oxygen electrode.

Q: What is the external diameter of the ICP catheter tip?

[S. Lau]: The pressure sensor mounted on the Millar telemeters have a 2 French outer diameter. This is roughly equivalent to 660µm.

Q: What is the sensitivity of sensor system in Prof Philips work and how did they remove noise?

[S. Lau]: The Millar TRM56SP SNA/BP telemeter has an input range of 60 microvolts. In addition to the SNA electrode lead wires, there is a grounding electrode which is secured on the muscle to help minimize noise in the system.

[J. Phillips]: The Millar system overcomes many of the drawbacks of the older system and shows much higher sensitivity and stronger signal acquisition properties relative to the older system most notably for blood pressure. Noise is one of the major issues associated with those types of recordings (SNA) and, in our experience, nothing much has been so far done by the company to minimize it as far as the recording system is in concern. However, there are several practices which can be carried out by researchers performing the recordings, which can help eliminate the noise feeding into the recordings as much as possible. The most important source of noise is related to the implantation surgery itself. The electrodes must be stabilized inside the rats by suturing them to the adventitia of the aorta/renal artery, a proper contact between the nerve and the electrodes must be ensured and optimal insulation for both the nerve and the electrode using SILGEL must be provided. Although, these practices minimize the noise, this issue is still sometimes unavoidable during the recordings. For this reason in our experiments all recordings were obtained during rats’ sleep cycle and major movement artifact, if present, were excluded from the recordings while analyzing the data.
Q: Would be possible to measure the activity of other nerves then RSN?

[S. Lau]: It is possible to measure the activity of other nerves using the Millar SNA+BP telemeter. Telemetry recordings of renal, lumbar and splanchnic nerve activity has previously been published (Stocker, S. D., & Muntzel, M. S. (2013). http://doi.org/10.1152/ajpheart.00173.2013). However, one must consider the accessibility of the nerve as well as the size of the nerve recordings as the Millar TRM56SP telemeter is designed for sympathetic nerves, and the recording range may not be suitable for some parasympathetic nerves, e.g. vagus nerve.
If you have additional questions for Dr. Fiona McBryde or Prof. Jacqueline Phillips regarding content from their presentations or wish to receive additional information on the Millar Telemetry system, please contact Dr. McBryde, Dr. Phillips or Dr. Sandy Lau from Millar by email:

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