## ELECTRODE DESIGN

- Helical-wire hook electrodes were made from sixty-five strands of thin 316LVM stainless-steel wires (0.001 inches in diameter each) insulated with Teflon (Med-wire 65, Cooner Wire Co, Chatsworth, CA, USA).
- A lead was formed into a helix near the stimulating tip by wrapping the lead 10 times around a 21-gauge needle with a tension of 2 to 3 kg.
- The stimulating tip consisted of one and a half cm of exposed wire, which was also wrapped into a helix and bent back to form a hook.
- The electrodes were sterilized, together with an insertion rod, which is put into the needle and advanced to the helical area of the electrodes; the rod was designed to ensure that the electrode tip stays where it is placed in the needle.

## ANIMAL PROTOCOL

- The protocol was approved by the Ethics Committee for Research on Animals, of the Banat University of Agricultural Sciences and Veterinary Medicine, Timisoara, Romania (Ethics Committee Approval No. 73 / 30$^{th}$ of January 2019, and Project Approval, from Romanian Veterinarian Authority No. 5 / 5$^{th}$ of February 2020).
- The protocol followed the Association for the Assessment of Laboratory Animals Care Guide for the Care and Use of Agricultural Animals in Research and Teaching.
- Three healthy Gottingen Minipigs (females, 8 months of age, 20 -30 kg; Ellegaard Minipigs, Dalmose, Denmark) were used in the study.
- Prior to surgery, the minipigs were acclimated for six months to remain in a study cage.

## ANESTHESIA

- A Duragesic (Fentanyl transdermal system) patch (100 ug/kg patch) was applied 24 hours prior to surgery.
- On the day of surgery, Azaperon 1.5 mg/kg IM and Atropine 0.05 mg/kg IM were administered prior to anesthetic induction.
- For antibiotic coverage, a single shot of Cefuroxime and Metronidazol was given.
- A 10 Ch Foley catheter was placed into the bladder.
- General anesthesia was induced with a combination of Xylazine (2 mg/kg), Propofol (10 mg/kg) and Ketamine (15 mg/kg) intravenously, and maintained by Sevoflurane 1.5%–2.5%.

- Sodium chloride 0.9% solution was administered at a rate of approximately 10 -11 ml/kg/h for the first hour, and then approximately at 5-6 ml/kg/h, for the balance of the surgical procedure.
- Depth of anesthesia was assessed by monitoring skeletal muscle tone, ocular reflexes and respiratory rate.

**SURGICAL INDUCTION OF A SACRAL SPINAL CORD LESION**

- The skin was shaved and sterilized.
- With the minipig in prone position, a midline 15-cm incision of the skin was conducted over the lower back with muscles separated along the midline, followed by the preparation of the dorsal spinal elements.
- A two-level-laminectomy ($L_5$, $L_6$ and partially $S_1$) was performed.
- The longitudinally cut dura exposed the spinal cord and the lumbo-sacral roots.
- The plan was to achieve a complete sacral spinal cord lesion by resecting the conus medullaris, but it was difficult to assess intraoperatively where the conus medullaris ended and the terminus filum (terminal thread) started.
- Only a limited conus lesion was produced, that included the $S_1$ and $S_2$ levels, but spared $S_3$-$S_5$ levels (this limited conus medullaris lesion was only determined during autopsy).
- The dura and skin were then closed in layers.

**PLACEMENT OF THE BLADDER WALL ELECTRODES**

- A subumbilical-suprapubic midline abdominal incision was conducted with the abdominal rectus muscle being separated along the midline, and the peritoneum was incised in order to expose the urinary bladder.
- The ureters were identified in the posterior parietal peritoneum, just over the common iliac artery, and dissected down to their insertion into the bladder wall.
- Two previously sterilized bilateral sets of bipolar electrodes were implanted.
- The caudal set was implanted adjacent to the bladder neurovascular bundle and 3 mm ventral to the ureters.
- The second bipolar set was implanted 1.5 cm directly rostral to the first set.
- In the first minipig, the electrodes were implanted in the superficial bladder wall with a 14-gauge needle and were further secured with two 3-0 polypropylene sutures.
- The electrode leads were looped in the abdominal cavity to reduce the risk of tension on the electrode stimulating tip.

- A risk of perforating the thin bladder wall was determined in the first animal, therefore the remaining two animals were implanted in the same locations, but with imbrication of the serosa around the electrode using sutures.
- Following implantation, a trocar was used to extend the electrode leads under the skin to an exit site on the back of the neck.
- To protect the external electrode leads, they were sutured to the skin at several locations, and a backpack was used with a securing belt placed under the animal.
- One to four knots on the ends of the leads identified the implant locations on the bladder wall.
- The abdominal and trocar incisions were closed.

**POSTOPERATIVE CARE**

- Twenty-four-hour surveillance was used to monitor the health of the animals during four weeks of follow up.
- Buprenorphine hydrochloride (0.01mg/kg, IV) and Perfalgan (paracetamol solution 100 mg/kg, IV) was administered in a 500 ml saline solution.
- The use of analgesics was continued for three days or until the animal returned to a normal husbandry schedule and appeared free of pain.
- For post-operative bladder emptying, a permanent Foley catheter and a urine bag were used, with the catheter tube taped to the tail.
- Intermittent catheterization could not be used because the animals had preserved perineal and anal sensations, making catheterization painful and difficult.
- The volume of urine in the Foley bag was monitored and a urine strip test was used to assess urinary tract infections (Combur 10 test, Roche, Vienna, Austria).

**ELECTRICAL STIMULATION**

- Two isolated stimulators (Model SD-9, Grass Inc, Quincy Mass., USA) were connected to the implanted electrodes as two sets of bipolar electrodes.
- The stimulation current was marked on the manual dial, which was determined using two electrodes in a normal saline bath and measuring the current with a 100-ohm resistor and an oscilloscope and applying Ohm's law.
- The stimulators were synchronized for simultaneous pulses.
- Stimulation tests were conducted with 400 μs pulses at 40 Hz stimulating frequency.

- Stimulation tests were conducted by increasing the current every few seconds, or by using a single constant current for 10 to 20 sec.
- Direct bladder wall stimulation was conducted with the bladder previously filled with 150-250 ml of saline solution.
- Adverse effects on skeletal muscles or animal discomfort were assessed with a rating scale of none, slight, moderate, and strong.
- The current was reduced or stopped if the animal demonstrated discomfort or excessive leg movement.

**URODYNAMIC MONITORING**

- Urodynamic testing was conducted with either the Foley catheter or after placement of a two-channel urodynamic catheter (Urodynamic 10 Fr, Laborie Inc) and a rectal balloon catheter.
- During autopsy the electrode locations were photographed, and extraction forces were measured by tying a string from the lead to a beaker and measuring the weight of water needed for dislodgement.

**AUTOPSY**

- During autopsy, the extent of the sacral spinal lesion and electrodes were evaluated.
- Electrode extraction force was measured in Newtons with a beaker tied to the lead and by the weight of water added to cause dislodgement.