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A Surgical Procedure for Implanting Radio Transmitters in Axolotls (*Ambystoma mexicanum*)

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Surgical procedures in daily veterinarian activities are performed to repair organs and tissues, conduct diagnoses, and assist in population control or experiments. However, surgery can also be applied for species conservation. For instance, the surgical insertion of transmitters for radio-telemetry techniques can be useful in understanding the population dynamics and behavior of species (Samuel and Fuller 1996; Faccio, 2003; Forsythe et al. 2004; Daenzer et al. 2005; Rittenhouse and Semlitsch 2007; Rowley and Alford 2007). Telemetry aids in understanding an animal's movement patterns within its habitat and yields information on its needs and habits (Gourret et al. 2011; Rogers and White 2007).

Telemetry has primarily been used in mammals and birds (Arnemo et al. 1999; Mech and Barber 2002; Habib et al. 2014). However, this technique has been used in other taxonomic groups such as fishes, amphibians, and reptiles (Madison 1997; Mulcahy 2003; Rittenhouse and Semlitsch 2007; Long et al. 2010). Amphibians often present a challenge for telemetry due to their small size and low weight.

External and internal transmitters have been used in amphibians. External transmitters do not require a surgical procedure and have the advantage of a long antenna, which enables broader distance detection (Heyer et al. 2001). Nevertheless, external transmitters require harnesses for support that could have several disadvantages and could compromise the results of the studies. Harnesses increase the chances that the organism becomes stuck between branches, and they can generate problems in locomotion, respiration, reproduction, thermoregulation, and visibility to predators (Reinert and Cundall 1982; Heyer et al. 2001; Muths 2003; Heemeyer et al. 2010). Harnesses can also generate skin problems such as abrasions (Goldberg et al. 2002), erythema, ulceration, nodules and papules (Weick et al. 2005; Caryn 2009; Long et al. 2010). A disturbance in the skin antimicrobial peptides can leave the animal susceptible to bacterial or fungal colonization (Chinnadurai and Lauren 2014). This makes amphibians particularly sensitive to environmental perturbations and cutaneous injuries (Pessier 2002; Daenzer et al. 2005).

The advantage of internal transmitters is to reduce modifications to the organism's behavior and development (Madison 1997; Rittenhouse and Semlitsch 2006; Long et al. 2010). Different protocols for the implantation of transmitters have been established and implemented for some amphibian species (Madison 1997; Rittenhouse and Semlitsch 2006; Peterman et al. 2008). For example, a procedure for the implantation of a 116-g transmitter in the large stream salamander *Cryptobranchus alleganiensis* (length 450–560 mm; weight 445–810 g) has been described by (Stouffer et al. 1983). In this case, the transmitter was introduced in the left side of the mid-ventral line of the abdominal region in animals anesthetized with tricaine methanesulfonate. A protocol for smaller salamanders such as *Dicamptodon tenebrosus (*156–233 mm; weight 39–72 g) was developed by Colberg et al. (1997). Here, a 2.5-g transmitter was implanted in the mid-lateral left flank in animals anesthetized with benzocaine.

However, internal transmitters also have some disadvantages such as a lower detection range (usually less than 50 m) and a shorter battery life. Another disadvantage is the surgical procedure, which increases the possibility of death due to anesthesia or post-surgical infections (Colberg 1997; Caryn 2009; Long et al. 2010). Surgical procedures also reduce the amount of tracking time because the organism needs a recovery period after surgery (Stouffer et al. 1983).

Therefore, appropriate transmitters must be chosen according to the physical and behavioral characteristics of the organisms. For example, external transmitters are recommended for arboreal frogs because they have larger home ranges (Heyer et al. 2001), whereas internal transmitters are more frequently recommended for aquatic and underground species (Heyer et al. 2001).

The Axolotl (*Ambystoma mexicanum*) is an aquatic and neotenic salamander with a maximum length of 300 mm and weight of 110 g (Armstrong et al. 1989), and is endemic to Xochimilco in the Valley of Mexico. This species is considered Critically Endangered (Zambrano et al. 2010a) because its population has decreased in the last decade, leading to its predicted extinction in the wild by 2019 (Zambrano et al. 2007). The primary causes for Axolotl population reduction include habitat degradation, the presence of invasive species such as carp (*Cyprinus carpio*), and Tilapia (*Oreochromis niloticus*), and reductions in water quality (Zambrano et al. 2004; Zambrano et al. 2007). There is currently a conservation program aimed at generating Axolotl refuges in canals within of its current range (Zambrano et al. 2010b). To evaluate these refuges, it is important to understand Axolotl behavior, habitat preference, and daily distribution. Radio telemetry represents an important method of evaluation (Ayala 2012). Because it is an aquatic amphibian, we decided to implant an internal radio transmitter following the suggestions of Heyer et al. (2001).

In this paper, we describe in detail the surgical method for the implantation of a radio transmitter in *A. mexicanum.* We also discuss similarities and differences with other protocols such as that proposed by Colberg et al. (1997). This surgical procedure aims to reduce potential infections, dehiscence, pain, complications, and death, looking for a full recovery in a shorter period of time, because the size of *A. mexicanum* limits the size and weight of the battery of the transmitters (Forsythe et al. 2004).

Surgical Procedure and Results

We developed a surgical procedure for the coelomic implantation of radio transmitters in 22 Axolotls. Fourteen Axolotls were obtained from the Ecological Restoration Laboratory at the Institute of Biology from the National Autonomous University of Mexico (Laboratorio de Restauración Ecológica del Instituto de Biologia [LREIB-UNAM; permit FAUT 0112]), and eight individuals were obtained from the Chapultepec Zoo, Mexico City. At the time of the procedure, all organisms were clinically healthy, with a size larger than 200 mm and weighed ≥ 53 g, and each had a subcutaneous microchip for identification.

Based on previous studies, radio transmitters should not exceed more than 10% of the total weight of the organism (Blomquist and Hunter 2007; Rowley and Alford 2007); other studies recommend not more than 5% (Wilson and McMahon 2006). For our procedure, the radio transmitters are oval shaped and weigh 1.2 g, less than 3% of the total weight of the Axolotl. We used two transmitter models: Telenax, Playa del Carmen, Quintana Roo, Mexico (TxB-0031) and HWSC Wildlife Materials, Murphysboro, Illinois, USA (SOPI-2011). Both transmitters had a battery life of 10–12 days. To implant Telenax transmitters, it was necessary to cover the wires with methyl methacrylate and to polish them to protect the internal organs. This increases the weight of the transmitter and reduces the duration of tracking. Therefore, in the second set of Axolotls, we decided to use HWSC Wildlife transmitters, which have the wires encapsulated within the transmitter during its manufacture.

Surgical area description.—The wet conditions of the area, and the great absorption capability of amphibians skin requires a short surgical time with an efficient hydration level of the organism (Gentz 2007). Therefore, the surgical area was divided into four progressively sterilized restricted sections: black, grey, white and recovery (Olfert et al. 1993). The black area is the common area; in this section, sterility is not the priority. This area includes a water container for anesthesia induction as well morphometric and biomass measurements. The grey area is used to prepare the organism for surgery. This section requires a medium level of sterilization because the abdominal region of the Axolotls is cleaned and disinfected in this section. The white section requires the highest level of sterilization. The radio transmitter is implanted in this section. Finally, the recovery area consists of a water container with oxygen pumps to help the Axolotl's recovery; therefore, it does not require a high sterilization standard (Solis et al. 2009). Before starting the procedure, all transmitters were disinfected by submerging them in chlorhexidine (75%; Nolvasan, Fort Dodge Animal Health) for 30 minutes. Afterwards, they were rinsed with sterile saline solution (Gray et al. 2005).

Anesthesia.—Prior to surgery, the study animals spent four hours fasting to prevent regurgitation. We began the procedure using Auralyt (Wyeth Labs, benzocaine) and later changed to isoflurane. Both of the anesthetics were diluted with water. The Auralyt doses were applied as follows: 165 mg/L; 220 mg/L; 300 mg/L (Wright 1996) and 317 mg/L. At concentrations of 300 mg/l and 317 mg/l of Auralyt, induction took 23 min, and the recovery time was 25 min. Using lower doses of this anesthetic, the induction of the animal was never reached. In the first surgeries, procedures were completed in 20 min; however, in the subsequent surgeries, experience helped reduce this period to only 12 min. The complete protocol, including the preparation of the animals for anesthesia to complete recovery, lasted approximately 80 minutes. Unfortunately, Auralyt was discontinued in Mexico; thus, we developed a second protocol with isoflurane (3 ml/L of water; Stetter et al. 1996; Wright 2001b). This anesthetic requires a hermetic sealing of the container to achieve induction, which was complete in 27 min; the associated time of recovery was approximately 44 min.

To ensure that the anesthetic procedure has been successful, the animal must remain static in a dorsal decubitus position and be unresponsive to physical stimuli. In procedure for both anesthetics, if the Axolotl continued to show movement, we applied three drops of non-diluted anesthetic (Isofluorane 100%; benzocaine 10%) on moistened gauze. The gauze covered the head and gills, considering that it is possible to use both anesthetics directly as mentioned in Mitchell (2009).

Surgical implantation.—After anesthesia, the Axolotls were weighed, measured, and placed in the lateral left decubitus position. In this position, the abdominal area was disinfected with a solution of chlorhexidine 75% (Wright 2001c; Harms 2005; Gentz 2007) and was rinsed with sterile saline solution to reduce skin irritation (Harms 2005) and losses to tissue integrity (natural skin mucus). Then, the animal was moved to the white area and placed on wet sterilized blankets soaked in saline solution. A no. 15 scalpel (Wright 2001c; Gentz 2007) was used for the surgery, which begins with an incision of 8–10 mm in the skin located in the right caudal quadrate, 1 cm from the right hind limb. This is a safe region for the incision because the stomach and the duodenum are located on the left side of the body (Wright 2001a). After the incision was made, scissors can be used to make a wider opening in the coelomic cavity (Gray et al. 2005). To preserve tissue integrity, skin manipulation should be avoided.

During surgery, the Axolotls were irrigated with a 0.9% physiological saline solution every three minutes to prevent desiccation and maintain osmotic balance on the skin (Harms 2005; Gentz 2007). During this time, the incision was covered with gauze to avoid contamination. The radio transmitter was inserted into the coelomic cavity in the caudal region (Daugherty and Sutton 2005) near the right abdominal wall (Fig. 1). In this area, the transmitter does not interfere with the function of the internal organs or with the behavior of the animal (Amlaner and MacDonald 1980; Wright 2001a).

Fig. 1. Axolotl under radio transmitter implantation surgical procedure.

Once the transmitter had been inserted, the coelomic cavity and the wound were rinsed with enrofloxacin 5 mg/kg (Wright and Whitaker 2001) and meloxicam 0.2 mg/kg to reduce infection risk, inflammation, and pain. After rinsing, the incision was closed with stitches placed 3 mm apart in a unique plane using polyglactin 3-0 (Vicryl, Ethicon, Somerville, New Jersey) (Colberg et al. 1997; Gray et al. 2005; Tuttle et al. 2006; Gentz 2007). Polyglycolic acid 3-0 can also be used as a suture material (Dexon United States Surgical, Norwalk, Connecticut) (Gentz 2007).

These sutures cause low inflammation and reduce potential dehiscence (Colberg et al. 1997). Finally, following previous surgical protocols (Crawshaw 1998; Wright 2001c; Gentz 2007), we applied a layer of the surgical adhesive cyanoacrylate (Vet Bond, 3M) in eight Axolotls to ensure tissue repair and to isolate the incision from possible contamination in the recovery tank. Meanwhile, in the remaining Axolotls (14), this adhesive was not applied in order to compare the animals' recovery with different materials. We found that this adhesive material induced skin necrosis on these aquatic salamanders and resulted in an incomplete recovery. By contrast, none of the 14 Axolotls without this adhesive presented any infection or skin problems, showing a faster recuperation.

When the procedure was concluded, the Axolotls were placed in recovery tanks that were filled with water at 12–14°C (Alworth and Harvey 2007). The water was oxygenated with pumps to stimulate rapid recovery (Willette-Frahm et al. 1995; Wright and Whitaker 2001). After 12 h, the Axolotls presented normal behavior, suggesting that they had fully recovered.

To prevent post-surgery infections and pain, we applied a combination of two broad-spectrum antibiotics. Three 24-h baths of metronidazole at 50 mg/l and an oral administration of enrofloxacin 5 mg/kg (Quimobac, 5% Brovel oral solution) (Wright and Whitaker 2001; Hadfield and Whitaker 2005) were applied once per day for five days. To reduce inflammation and pain, meloxicam was provided once per day for three days orally at doses of 0.1 mg/kg (Bradley 2001). We developed this protocol instead of using the green malachite used in previous protocols (Colberg 1997) because it is only an antifungal and may produce unwanted consequences. Green malachite is considered to be a mutagenic, teratogenic and potentially toxic substance (Carpenter 2012). Axolotls remained under observation in tanks for seven days prior to being released in the environment to be tracked for 72 h.

During the tracking period, Axolotls were found in 94% of the attempts. They moved on average 2.8 m/h, showing preference for nocturnal activities, without a significant difference of movement among them (Ayala 2012).

Once the radio telemetry tracking was concluded, Axolotls were recovered and transferred to the laboratory for physical examinations. None of the Axolotls died, but they lost approximately 6% of their weight on average. Three of them, in which Vetbond was applied, presented dehiscence; other three Axolotls presented infection but no dehiscence; and finally one animal presented a 1-cm intestinal exposition. Although it seems these health problems did not affect movement patterns (Ayala 2012), this information should be considered when interpreting our results.

The recovered animals received medical treatment according their clinical situation. Those animals that showed dehiscence received treatment in the form of irrigating the skin with a 0.9% saline physiological solution and disinfection with chlorhexidine at 75%. The dehiscence area was repaired with two stitches with a 3 mm gap in a unique plane. Then, Axolotls were placed in a bath of water with metronidazole 50 mg/L for 24 h (Wright 1996). These baths were repeated in three cycles of 24/24 h. Those animals with infections were also medicated with metronidazole in the same type of baths treatment described above. Individuals were fully recovered in 15 days from both diseases.

The Axolotl that presented intestinal exposure was anesthetized following the isofluorane procedure described above. Afterwards the exposure area was cleaned with a 0.9% saline physiological solution, disinfected with chlorhexidine 75%, and the intestine was returned to the coelomic cavity. The abdominal wound was repaired with four stitches with a 3 mm gap. An intramuscular analgesic (meloxicam) was applied at a dose of 0.2 mg/kg, followed by an antibiotic (enrofloxacine) at a dose of 7 mg/kg. Finally, this animal was placed in metronidazole baths such as described above. This organism was fully recovered after 30 days.

A week after the experiment, all healthy animals gained weight up to their initial condition, suggesting that loss of weight was the result of field circumstances. A month after the full recovery, the radio transmitters were removed following the described protocol. These animals are kept in the colony for reproduction purposes and were not used for any other experiment.

Conclusions

Surgical protocols must consider the anatomy and physiology of the focal species. Here, we presented a detailed surgical process for *Ambystoma mexicanum* that includes pre and postsurgical care for the animals. We also provided solutions for problems resulting from surgery or poor recovery.

The protocol presented for surgical implantation in Axolotls can be considered as an update of Colberg et al. (1997). This protocol uses materials and anesthesia that speeds the recovery and reduces the infection probabilities, while also increasing the time range of detection for field experiments. Unlike the Colberg et al. (1997) procedure, we implanted the transmitters on the right side of the coelomic cavity. We consider it a safer option because the stomach of *A. mexicanum* is displaced to the left (Wright 2001a).

The use of cyanoacrylate as a surgical adhesive appears to generate skin problems in aquatic salamanders, since all of our test subjects with this adhesive developed problems after surgery. Additionally, it is important to provide a combination of antibiotics and painkillers for a more complete and rapid recovery following the surgical procedure.

A rapid recovery is essential considering the short battery life of small radio transmitters. In this study only 36% of the animals experienced infections or dehiscence despite the short period before they were released on the field after surgery, and all of them recovered properly after treatment when they returned to the colony. However, this percentage should be reduced in the future for tracking experiments. Based on our results, we recommend this surgical procedure for radio transmitters in ambystomatids and other salamanders when rapid recovery is needed. However, more studies to reduce the percentage of infections and the proper time of recovery are desirable. Finally, this method can be modified for other aquatic species with similar characteristics.

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