Ketterson / Nolan Research Group Collection

This document is part of a collection that serves two purposes. First, it is a public archive for data and documents resulting from evolutionary, ecological, and behavioral research conducted by the Ketterson-Nolan research group. The focus of the research is an abundant North American songbird, the dark-eyed junco, *Junco hyemalis*, and the primary sources of support have been the National Science Foundation and Indiana University. The research was conducted in collaboration with numerous colleagues and students, and the objective of this site is to preserve not only the published products of the research, but also to document the organization and people that led to the published findings. Second, it is a repository for the works of Val Nolan Jr., who studied songbirds in addition to the junco: in particular the prairie warbler, *Dendroica discolor*. This site was originally compiled and organized by Eric Snajdr, Nicole Gerlach, and Ellen Ketterson.

Context Statement
This document was generated as part of a long-term biological research project on a songbird, the dark-eyed junco, conducted by the Ketterson/Nolan research group at Indiana University. For more information, please see IUScholarWorks (https://scholarworks.iu.edu/dspace/handle/2022/7911).

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Anesthetic: Anesthetic is necessary during surgical implant placement. Traditionally our group used metofane to immobilize juncos in order to quickly insert two 5 or 10 mm implants beneath the skin (depending on sex). The metofane gave us time to place the implants properly since it was critical that they not fall out. Metofane has become unavailable in the United States, therefore we have switched to other anesthetics.

Isoflurane: Isoflurane is applied to a cotton pad at the bottom of a glass Erlenmeyer flask at the concentration of 200 µL per liter of flask capacity. This concentration produces a vapor pressure of ~4% in the flask. This is the same vapor pressure used in the approved anesthesia methods for Metofane. For the juncos, we use a 200 mL flask with 40 µL of isoflurane. The bird’s head is placed in the neck of the flask, well away from contact with the cotton ball with the liquid isoflurane. The bird is held in the flask for 40-60 seconds, until neck muscles relax and eyes close. In mice, this dosing produces general anesthesia in 57±21 seconds of exposure (Dr. Sanford Feldman, DVM, PhD, Director, Center for Comparative Medicine, University of Virginia, personal communication). This method produces a general anesthesia (relaxed neck muscles, closed eyes, elevated neck feathers, largely irresponsible to toe-pinch) that lasts for 1-2 minutes, and a recovery to full activity in an additional 1-2 minutes. If more time is needed for the operative procedure, birds can be re-dosed with an additional 20-30 seconds of exposure to the isoflurane. Because of the high rate of vaporization of isoflurane, the cotton pad must be re-dosed after every 3-4 birds.

Halothane: A 15% solution of halothane in light mineral oil is prepared (150 µL halothane, 850 µL mineral oil) by vortexing. This solution is applied to a cotton ball or gauze square at the end of a small screw-top vial. The mineral oil suspension prevents immediate vaporization of the halothane and maintains a vapor pressure of halothane appropriate for anesthesia. This mixture is made fresh every day that anesthesia is used, and is kept in the dark when not in use. The bird’s head is placed in the neck of the vial, well away from contact with the cotton ball with the halothane mixture. The bird is held in the flask for 1-2 minutes, until heart and breathing rates slow, neck muscles relax and eyes close. This method produces a general anesthesia (relaxed neck muscles, largely irresponsible to toe-pinch) that lasts for 2-5 minutes. If more time is needed for the operative procedure, birds can be re-dosed with an additional 30-45 seconds of exposure to the halothane mixture.

Current anesthetic protocol for surgical implant placement: Isoflurane is used with a vaporizer according to manufacturer protocol to ensure constant anesthesia during the implant process.

Surgical implantation protocol
After the bird is anesthetized, place it on the implanting surface on its right side. Gently position the left wing up over the bird’s back, and stretch the feet downward. (It helps to have two people; one to hold the bird in position and one to place the implant.)
Sterilize the skin surface using an ethanol wipe. Using sterilized surgical scissors, cut a small hole (2-3mm) in the loose skin between the knee and hip joint, where it has moved away from the body wall as the leg is straightened.

Pick up the implant by one end using sterilized forceps, and briefly rinse it with ethanol to remove any grains of crystalline hormone from the outer surface. Once it has dried, insert one end of the implant into the small hole, typically with one end pointing towards the hip joint. A blunt probe can be used to push the implant completely under the skin, although be careful not to tear the opening in the skin.

Once the implant is completely under the skin, it can be moved and positioned by gently pushing and rolling on the skin surface with a moistened cotton swab. If possible, the implant should be moved into position high on the left flank, parallel with the spine/back feather tract, and roughly perpendicular to the hip joint. In general, the implant should be moved away from the incision site so as to minimize the chances that it will fall out once the bird is released.

Once the implant is in position, use forceps to carefully remove any tiny pin-feathers that may have been pushed into the incision site, as these will prevent clean healing of the wound if they are left under the skin. Use your fingers to pinch the edges of the incision together, and hold for ~30 seconds. If the incision is large, a drop of veterinary adhesive (such as VetBond) can be applied to the site. Once this has dried, the bird can be taken off anesthetic. Once the bird has fully woken up, and the surgery site has been checked, the bird can be released.

**Trochar implantation protocol (non-surgical)**
The implants are inserted beneath the skin along the left flank by inserting the implants with a trochar syringe. The trochar needle is 12-gauge, 1.5 inches in length. The bird is held in hand for the procedure, which typically lasts less than 6 minutes. After application of topical anesthetic (5% Lidocaine ointment) and a topical antiseptic (iodine (0.75%) or chlorhexidine (2%)), and allowing time (3 min) for the topical anesthetic to take effect, the implant is inserted subcutaneously by injection along the inner thigh, parallel to the thigh muscle when the leg is held extended, between the muscle and the skin (the body cavity is not entered). The implant can then be positioned along the flank by using gentle pressure through the skin in the same way described in the surgical implantation protocol.

**Implant removal**
General anesthesia is not required for this procedure, although topical anesthetic/antiseptic may be used (as above in the trochar implantation protocol). A small incision is made with sterilized surgical scissors directly over one end of the implant. The implant can be pushed from the other end through the skin towards the incision. The implant is grabbed by that end with forceps, removed from the bird, and stored in a small Eppendorf tube for later weighing and verification. The incision site should be pinched together with the fingers and held for 30 seconds, or sealed with VetBond if desired.